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LOCAL ALLERGIC REACTION OF PREVIOUSLY VACCINATED SKIN DURING ASIAN INFLUENZA

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IN NOVEMBER, 1957, a 32-year-old white man was inoculated intradermally with Asian influenza vaccine. Inoculation was attempted in the skin of the flexor aspect of the right forearm, but mishap led to inadequacy of the dose. A full dose, presumably 0.1 c.c., was therefore injected into the left forearm. There were no reactions.

In March, 1958, during the epidemic appearance of Asian influenza, the patient became ill with high fever and acute bronchitis. He recovered in five days. A roentgenogram of the chest revealed no abnormality. The blood leukocyte count was not elevated. However, there was some left shift in the differential count at the height of the illness, but this returned to normal with recovery. The illness resembled the other cases of influenza being seen in the hospital at that time.

On the initial day of illness, there appeared two erythematous areas at the sites of inoculation of vaccine four months earlier (Fig. 1). These became slightly indurated and tender. The area involved was larger in the left forearm, which had received the full dose of vaccine. The erythemas subsided with recovery from the illness.

It was concluded (though unproved) that the patient had contracted Asian influenza, despite intradermal inoculation of prophylactic vaccine four months earlier, and that the illness had caused specific reactivity of the locally inoculated skin.

COMMENT

That a local immunity may exist in the absence of a general immunity has been recognized since early studies with experimental erysipelas in rabbits.

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The tissues become allergic during the immunizing infection, as indicated by prompt and vigorous reactivity to the specific antigenic stimulus. Later observations extended the phenomenon to other bacterial infections.[1]

A similar local immunity has been observed in viral infections. Four days after intradermal injection of vaccinia virus in rabbits, tissue extracts from the inoculated area contained two to ten times as much antibody as blood or other tissue extracts.[2] Intranasal vaccination of mice with influenza virus gave a ratio of antibody in the respiratory tract to that in serum of 1:20, compared with a ratio of 1:250 following parenteral vaccination.[3] The local production of antiviral antibodies in inoculated tissue was shown for poliomyelitis by Morgan,[4] and for equine encephalomyelitis by Schlesinger.[5]

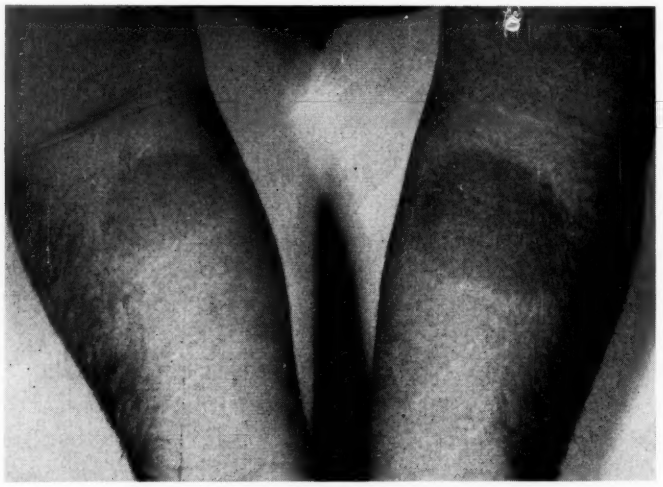

Fig. 1.—Local allergic reaction during Asian influenza, occurring at sites of intradermal inoculation of Asian influenza vaccine four months previously.

Increased reactivity of the skin to injection with live or inactivated virus is demonstrable in persons or animals that have recovered from a variety of viral diseases, including smallpox, vaccinia, psittacosis, lymphogranuloma, trachoma, influenza, herpes, mumps, swine fever, and contagious ecthyma of sheep.[6, 7] Beveridge[7] suggests that persisting allergy to viruses need not be associated with the continued presence of neutralizing antibody, since repeated reinoculations may produce a full "primary" type of reaction.

When the above reported patient became ill with influenza, a local allergic response occurred in skin areas inoculated four months previously. Since monovalent Asian influenza vaccine (a variant A strain) had been used, and since Asian influenza was epidemic in the community at the time, it is assumed that a specific allergic response was elicited by the disease in the locally immunized skin. I have found no reference to this phenomenon, in which the local reaction was produced by the disease after earlier inoculation. It is reminiscent of the Schwartzman phenomenon.[8] In that experiment, intravenous injection of homologous culture filtrate, eight to thirty-two hours after

inoculation of bacteria or culture filtrates into the skin of rabbits, resulted in gross hemorrhage and necrosis in the inoculated skin. The Schwartzman phenomenon is sharply limited in its time of occurrence, however, apparently depending on the stage of the initial inflammatory reaction. The presently reported observation suggests, rather, a "skin test" in reverse (that is, produced by the disease in previously inoculated skin) and is probably similar in its mechanism to the skin reactions obtained on specific inoculation of sensitized persons. It may bear analogy with the "fixed eruptions" produced in some persons by ingestion of specific drugs.[9]

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ENDOCRINE FUNCTION IN BRONCHIAL ASTHMA AND HAY FEVER

A Controlled Study of 17-Ketosteroid and 11-Oxysteroid Excretion

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UNCOMPLICATED bronchial asthma is noteworthy for the high incidence of remissions induced by cortisone therapy, without which some patients are unable to maintain reasonable health. Spontaneous remissions are also frequent following puberty, surgical operations, alteration of life situations, and the removal of specific allergic factors. Although clinical manifestations of endocrine dysfunction are seldom noted in untreated patients with chronic active bronchial asthma, some reports have described a reduction of 17-ketosteroid excretion in these patients,[1, 2] as well as decreased adrenocortical function.[1, 3, 4] The present study is part of a combined medical and psychiatric evaluation of the pathogenesis of bronchial asthma, which has included serial observations of pulmonary function, psychologic testing, psychiatric interviews, and hormone urinary excretion in ambulatory nonhospitalized patients and preselected age- and sex-matched adult controls. Observations of endocrine function will be reported here, amplifying a preliminary report.[5]

METHODS

Four groups of observations, based upon the presence or absence of an allergic disorder were made in this study. The control group was selected from available volunteer personnel, in many instances doctors, nurses, students, or hospital aides whose medical history was known to be free of allergy, endocrine disorder, or any type of chronic disease. A second group was composed of hay fever patients who were in remission at the time of the urine collections. None of these patients had previously shown any evidence of obstructive pulmonary disease (asthma) by clinical examination or timed vital capacities. A third group was composed of patients with mild asthma who had only intermittent respiratory distress and required scanty daily nonsteroid medication, usually an ephedrine preparation or isopropylarteronal by inhalation. These patients had required neither hospitalization for asthma nor steroid medication within the past three years. A fourth group was composed of patients with severe bronchial asthma who had daily respiratory distress causing partial incapacity and who required regular nonsteroid medication. All patients in this latter group had required hospitalization on one or more occasions during the three

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years preceding the study or had required steroid therapy for some time during this period. None, however, had received ACTH or steroids for at least two weeks prior to study. The physical data on control volunteers and patients are summarized in Table I. The age range of the asthmatic patients was slightly older than that noted in the control group, except for the group of female patients with severe asthma.

TABLE I. PHYSICAL DATA ON HEALTHY CONTROLS

	FEMALES				MALES			
CATEGORY	NO.	AGE RANGE AND MEDIAN (YEARS)	HEIGHT RANGE AND MEDIAN (INCHES)	WEIGHT RANGE AND MEDIAN (POUNDS)	NO.	AGE RANGE AND MEDIAN (YEARS)	HEIGHT RANGE AND MEDIAN (INCHES)	WEIGHT RANGE AND MEDIAN (POUNDS)
Healthy volunteers	6	22-30 29	62-67 64	115-138 120	9	22-46 21	65-74 72	140-195 180
Hay fever	3	23-33 29	62-65 62	118-120 118	3	28-47 33	72-74 72	180-220 185
Mild asthma	4	28-43 38	62-68 64	130-155 140	3	18-45 41	69 --	160-165 --
Severe asthma	4	25-38 30	60-66 64	120-130 125	8	28-50 40	64-75 67	150-215 165
Total cases = 40	17				23			

Each patient prior to study was matched as closely as possible with a healthy volunteer of same sex, age, and physical habitus, and twenty-four-hour urine collections were performed simultaneously during periods when both the patient and the control were carrying on their usual activities. The completeness of collection was verified by creatinine determinations.

Analyses of urine specimens were carried out for total 17-ketosteroids by a modification of the method of Drekter and McGavack,[6] for the glucuronides of androsterone and etiocholanolone by a modification of the method of Rubin and associates,[7, 8] and for 11-oxy-17-ketogenic steroids, by the method of Wotiz, Lemon, and Marcus.[9]

The results have been reported as the group means of individual means from each case, representing at least five twenty-four-hour collections for total 17-ketosteroids and a somewhat smaller number of analyses in the case of the individual 17-ketosteroids and total 11-oxysteroids.

The severity of the asthmatic state was estimated in all patients from a daily score sheet maintained by the patient, by which the patient's limitation of activity and medication requirements could be estimated.[10] Timed vital capacities (expirograms) were performed at weekly intervals on most asthmatic patients. In order to eliminate any possible bias, the results of the observations in any one category—clinical, hormonal, or subjective (score sheet)—were not compared with those obtained in any other category until the hormonal studies were complete.

The results of the urine analyses have been statistically evaluated for significance of differences between group means, utilizing the usual "t" test of

significance. The variance within each group mean was compared to that of each of the other group means whose significance of difference was to be assessed; the ratio of these two variances was calculated. Using the method and tables prepared by Edwards,[11] the homogeneity of variance was calculated. Where variance differences between group means were insignificant, the usual formula for calculation of "t" values and probability of occurrence by chance (p) was utilized. Where variance proved to differ significantly between group means, the altered value of "t" necessary for a significant level of difference between means was calculated and utilized for determination of the p value.

EXPERIMENTAL RESULTS

1. *11-Oxy-17-Ketogenic Steroid Excretions.*—The results of 11-oxysteroid analyses are shown in Table II. Although slightly lower mean values characterized the asthmatic patients, compared to either hay fever or control groups, none of the observed values was abnormal; nor were the differences between the groups of any statistical significance. No significant relationship could be established between the 17-ketosteroid and 11-oxysteroid excretions.

TABLE II. TOTAL URINARY 11-OXY-17-KETOGENIC STEROID EXCRETION IN ASTHMATIC AND NONASTHMATIC SUBJECTS

GROUP	NO. OF SUBJECTS	NO. OF SAMPLES	MEAN OF INDIVIDUAL SUBJECTS ± S.E. MILLIGRAMS PER TWENTY-FOUR HOURS	P VALUE OF DIFFERENCE FROM ( )
Female				
Control (C)	5	5	2.6 ± 0.3	------
Severe asthma	4	4	2.0 ± 0.4	Insignificant (C)
Mild asthma	4	4	1.8 ± 0.3	Insignificant (C)
Hay fever	3	3	2.3 ± 1.0	Insignificant (C)
Male				
Control (C)	4	4	3.8 ± 1.0	------
Severe asthma	4	4	2.9 ± 0.5	Insignificant (C)
Mild asthma	1	1	(3.6) --	Insignificant (C)
Hay fever	3	3	6.2 ± 1.4	Insignificant (C)
Total	28	28		

TABLE III. TOTAL URINARY 17-KETOSTEROID EXCRETION IN ASTHMATIC AND NONASTHMATIC SUBJECTS

GROUP	NO. OF SUBJECTS	NO. OF SAMPLES	MEAN OF INDIVIDUAL SUBJECT AND S.E. MEANS (MG./24 HRS.)	P VALUE OF DIFFERENCE FROM ( )
Females				
Controls (C)	6	40	8.3 ± 0.9	----
Severe asthma	4	33	4.6 ± 0.5	0.02 – 0.01 (C)
Mild asthma	4	16	4.9 ± 0.5	0.05 – 0.02 (C)
Hay fever	3	17	11.0 ± 2.4	Insignificant (C)
Males				
Controls (C)	9	63	14.7 ± 1.2	----
Severe asthma	7	80	8.0 ± 0.9	0.01 (C)
Mild asthma	3	27	6.9 ± 1.3	0.01 (C)
Hay fever	4	15	17.9 ± 5.4	Insignificant (C)
Total	40	291		

2. *17-Ketosteroid Excretion.*—Table III shows the mean of individual subject means for all eight groups studied, together with results of analysis of variance, and the probability (p) that the observations might have been the result of chance. At least five determinations were used to calculate the mean in each case.

In both male and female asthmatic patients the 17-ketosteroid excretion was only a little more than one-half of that noted in healthy volunteers or in patients with hay fever. The asthmatic patients differed from the healthy controls by a highly significant probability, with chance occurrence restricted to 1 out of 50 to 100. Hay fever patients were not significantly different from the controls, even though their mean value was somewhat greater, because of the wide variance observed in their 17-ketosteroid excretion.

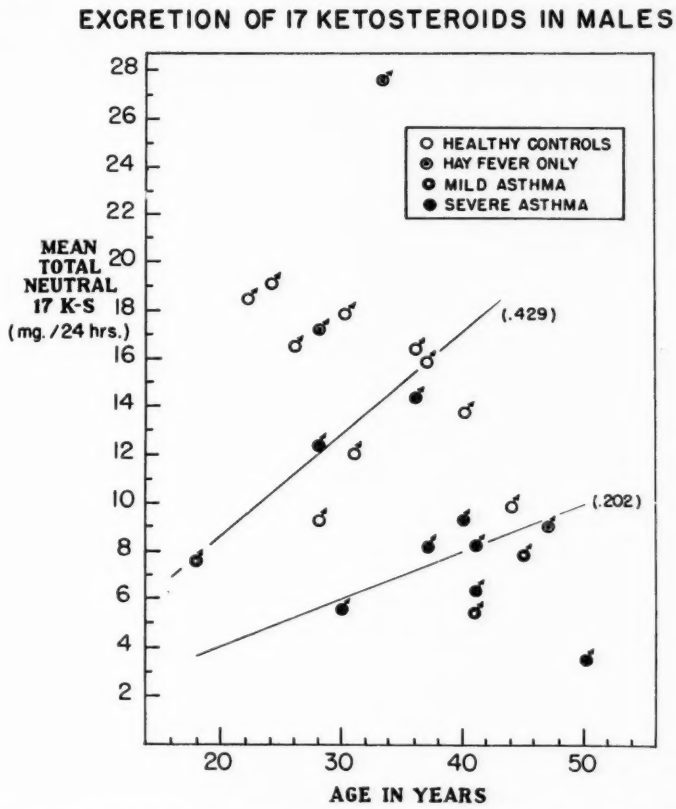

Fig. 1.—Relationship between 17-ketosteroid excretion and age in male subjects. The coefficients of regression have been calculated for healthy controls and hay fever patients combined (0.429) and for all asthmatic subjects (0.202).

The excretion of 17-ketosteroids varies widely according to age, whereas 11-oxysteroid excretion remains more nearly constant after infancy and throughout life.[12] When the means of individual patients' 17-ketosteroids are plotted against age, it can be seen that the lower values of the asthmatic patients cannot be attributed to the ages of the patients in the various groups (Figs. 1 and 2).

TABLE IV. URINARY GLUCURONIC ACID CONJUGATED ANDROSTERONE AND ETIOCHOLANOLONE EXCRETION IN ASTHMATIC AND NONASTHMATIC SUBJECTS

GROUP	NO. OF SUBJECTS	NO. OF SAMPLES	MEAN OF INDIVIDUAL SUBJECT MEANS AND S.E. (MG./24 HRS.)		P VALUE OF DIFFERENCE FROM ( )	
			ANDROSTERONE GLUCURONIDE	ETIOCHOLANOLONE GLUCURONIDE	ANDROSTERONE GLUCURONIDE	ETIOCHOLANOLONE GLUCURONIDE
Female						
Controls (C)	5	5	0.8 ± 0.1	2.0 ± 0.3	----	
Severe asthma	4	4	0.5 ± 0.3	1.6 ± 0.5	Insignificant (C)	
Mild asthma	3	3	0.5 ± 0.1	0.8 ± 0.2	Insignificant (C)	0.05-0.02 (C)
Hay fever	3	3	1.4 ± 0.6	2.0 ± 0.9	Insignificant (C)	
Male						
Controls (C)	5	5	1.3 ± 0.3	3.0 ± 0.5	----	
Severe asthma (S.A.)	3	3	0.8 ± 0.2	1.6 ± 0.5	Insignificant (C)	
Mild asthma	1	1	0.6 ± --	1.4 ± --	Insignificant (C)	
Hay fever	3	3	1.7 ± 0.2	3.5 ± 1.0	Insignificant (C)	Insignificant (C)
						0.05-0.02 (S.A.)
Total	27	27				

There is a wider spread of values in the case of the male patients, probably as a result of the varied contributions from testes and adrenal cortices in this group (Fig. 1). On the other hand, it appears that among female patients nearly all of the 17-ketosteroids are of adrenicortical origin, and perhaps for this reason there is better segregation of the means of female asthmatic patients from those of female volunteers (Fig. 2). The regression coefficient for individual means of both sexes is about 50 per cent less in the case of the asthmatic subjects, compared to the pooled values of controls and hay fever patients.

Application of the Chi-square test of significance to the eight categories of 17-ketosteroid observations resulted in a p value of .01 or less.

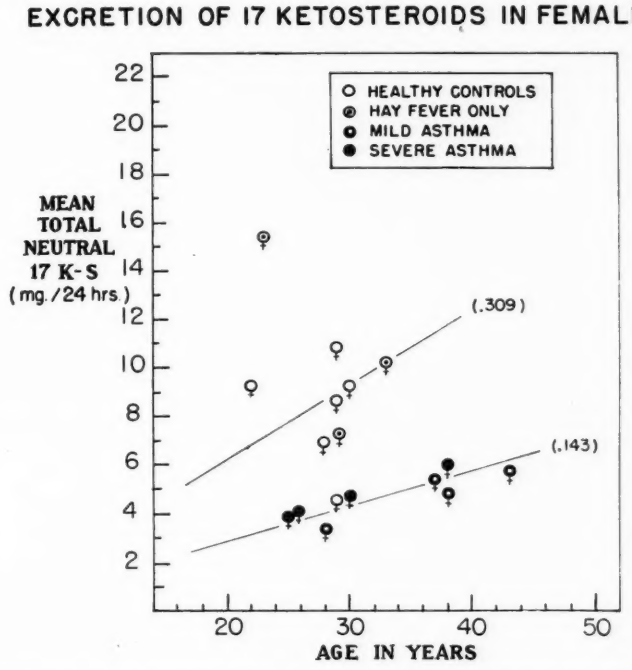

Fig. 2.—Relationship between 17-ketosteroid excretion and age in female subjects. The coefficients of regression have been calculated for healthy controls and hay fever subjects combined (0.309) and for all asthmatic subjects (0.143).

3. *Androsterone and Etiocholanolone Excretion.*—In an effort to ascertain which of the individual 17-ketosteroids were decreased in the urine of the asthmatic patient, aliquots of specimens were analyzed for androsterone and etiocholanolone glucuronide. These steroids comprise the major portion of glucuronide-bound urinary 17-ketosteroids.[8] As shown in Table IV, reduced androsterone and etiocholanolone excretions were noted in mild and severe asthmatic men and women, as compared to normal controls and hay fever patients. Because of the very low level of androsterone excretion and the small number of samples in each group, statistical significance could not be attached to these observations except for the excretion of etiocholanolone in two instances. The trend noted in total 17-ketosteroid studies appears, however, to be borne out by these analyses.

The ratio of etiocholanolone to androsterone remained unaltered throughout all male groups investigated (Table V). Among female subjects, this ratio was markedly reduced in hay fever and mild asthmatic patients, as compared to normal patients and those with severe asthma.

TABLE V. RATIO OF ETIOCHOLANOLONE TO ANDROSTERONE EXCRETION IN ASTHMATIC AND NONASTHMATIC SUBJECTS

GROUP	NO. OF SUBJECTS	ETIOCHOLANOLONE GLUCURONIDE (MG./24 HRS.) / ANDROSTERONE GLUCURONIDE (MG./24 HRS.) MEAN OF INDIVIDUAL SUBJECTS	P VALUE OF DIFFERENCE FROM ( )
Females			
Controls (C)	5	2.6	----
Severe asthma (S.A.)	4	3.8	Insignificant (C)
Mild asthma	3	1.6	0.05-0.02 (C)
Hay fever	3	1.5	0.05-0.02 (C) (S.A.)
Males			
Controls (C)	5	2.6	----
Severe asthma	3	2.0	Insignificant (C)
Mild asthma	1	(2.2)	Insignificant (C)
Hay fever	3	2.1	Insignificant (C)

4. *Alteration of Steroid Excretion and Pulmonary Function During Asthmatic Relapses.*—In seven patients (three male, four female) daily twenty-four-hour urine collections were obtained over a period of weeks or months, during which time the patients recorded the severity of their asthma by means of daily score sheets. Serial expirograms were obtained at intervals of twenty-four to seventy-two hours, depending upon the severity of the disease, with attention directed toward recording the minimum vital capacity during asthmatic relapse. The daily score sheet has been shown to reflect the severity of the asthmatic state as determined by expirograms.[10] The expirogram and score sheet data were accompanied by analyses for 17-ketosteroids and 11-oxysteroids for periods of months in these seven patients. No ACTH or steroid therapy was administered to these patients during the study period. Correlation coefficients were calculated for the expirogram values, score sheet results, and steroid analyses during repeated seven-day periods when all three sets of observations were available, with a single reference day selected arbitrarily for each set of calculations. A positive correlation was established for each day's observation if the daily observation of each pair of variables exceeded the mean for the series of observations of each variable or if both observations were below the mean.

In all female subjects and in three of four male subjects an inverse correlation between the score sheet tally and the expirogram was noted, which was significant at the $p = 0.01$ level or less, confirming previous work[10] (Table VI).

No significant correlation between score sheet results and steroid excretion was found, however, in these cases observed during a period of months. No constant relationship could be noted even between the trend of 17-ketosteroid

TABLE VI. SUMMARY OF CORRELATIONS NOTED BETWEEN TREND OF SCORE SHEET, EXPIROGRAM, AND STEROID EXCRETION

CORRELATED ITEMS	DAY OF OBSERVATION	PATIENT						
		C. ♂	B. ♂	KE. ♂	KU. ♀	G. ♀	W. ♀	S. ♀
Expirogram vs. score sheet	−3	+	− (0.0005)	−	− (0.0005)	− (0.01)	− (0.0005)	− (0.02)
	−1	+	− (0.0005)	−	− (0.0005)	− (0.0005)	− (0.0005)	− (0.01)
	Reference day	+ (0.02)	− (0.005)	− (0.02)	− (0.0005)	− (0.005)	− (0.0005)	− (0.01)
	+1	+ (0.005)	− (0.0005)	− (0.0005)	− (0.0005)	− (0.05)	− (0.0005)	− (0.01)
	+3	+ (0.01)	−	− (0.005)	− (0.0005)	−	− (0.0005)	−
11-Oxy-17-ketogenic steroid vs. score sheet	−3	−	+	−	−	−	+	+
	−1	−	+	− (0.05)	−	−	+	+
	Reference day	−	+	−	−	+	−	+
	+1	−	+	−	−	−	−	+
	+3	−	+	− (0.02)	− (0.05)	−	−	+
17-Ketosteroid vs. score sheet	−3	+	− (0.01)	−	+	+	+	+
	−1	+	− (0.02)	−	−	+	+	+
	Reference day	+	−	−	+	+	−	+ (0.05)
	+1	+	−	−	+	+	+	+ (0.02)
	+3	+	−	+	+	+	+	+ (0.02)
11-Oxy-17-ketogenic steroid vs. 17-ketosteroid	7-day period	−	+	+	+ (0.05)	−	+	+ (0.01)

Direction of correlation noted by plus and minus signs. Figures in parentheses indicate p value of significance of correlation, as determined by Chi-square test.

and 11-oxysteroid excretion in individual patients, although in most cases the values tended to rise or fall together. Thus, no evidence was obtained in this study that adrenocortical function was related to changes in the asthmatic state or pulmonary function.

5. *Effect of the Administration and Withdrawal of Cortisone and Prednisone.*—In the course of other studies in nonasthmatic female patients we have noted marked reduction in excretion of androsterone and etiocholanolone as long as cortisone or prednisone therapy is maintained.[13]

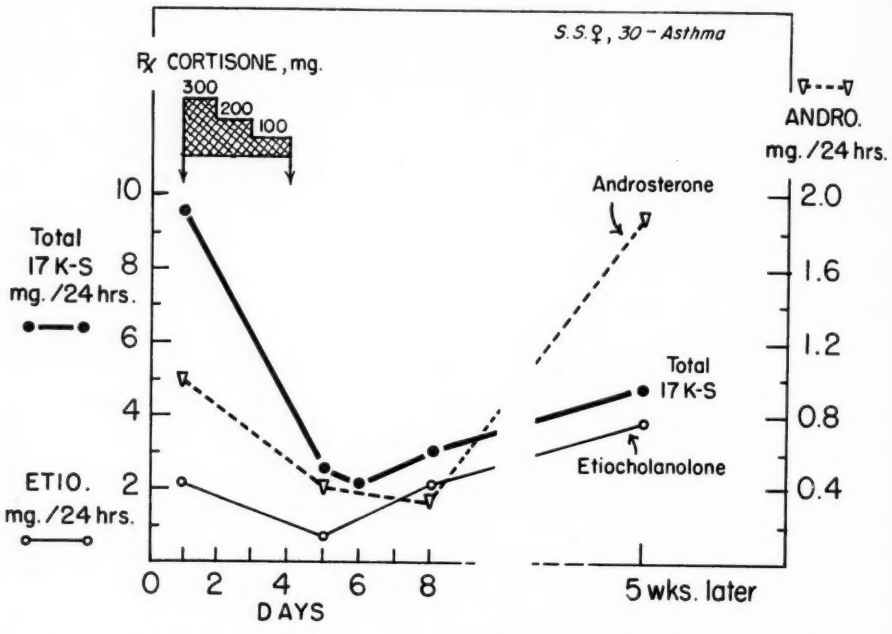

Fig. 3.—The effect of cortisone therapy on the total and fractional 17-ketosteroid excretion.

Serial observatios were carried out in a few of the female asthmatic patients to evaluate the influence of similar steroid therapy upon these urinary metabolites. Cortisone was administered in 300, 300, and 100 mg. total daily doses on consecutive days, followed by 5 to 20 mg. prednisone for one day or two days in some cases. On the days immediately following withdrawal of steroids, analyses were conducted to compare the excretion of urinary steroids with pretreatment periods. Excretion of 17-ketosteroids usually decreased during the treatment period, along with a marked drop in androsterone and etiocholanolone glucuronides. Excretion of androsterone-glucuronide fell to very low levels approaching the limits of sensitivity of the analytic procedure (Fig. 3). Within several days after termination of steroid treatment, urinary androsterone, etiocholanolone, and total 17-ketosteroid excretion had returned to pretreatment levels. Simultaneously, recrudescence of asthmatic symptoms and signs occurred in some patients.

DISCUSSION

The data presented here suggest that the asthmatic state, whether "mild" or "severe," is accompanied by a statistically significant reduction of 17-ketosteroid excretion in both male and female patients as compared to preselected matched healthy volunteers or to hay fever patients without asthma. The observed reduction of urinary androsterone and etiocholanolone glucuronide excretion in asthmatic patients supports this finding. In spite of the reduced 17-ketosteroid excretion, the mean total corticoseroid excretion of asthmatic subjects was not significantly reduced when these patients were compared to a control population. The administration of cortisone or prednisone accentuated the 17-ketosteroid deficiency of the asthmatic patient, probably through inhibition of ACTH secretion. These observations confirm several previously reported studies of 17-ketosteroid excretion in bronchial asthma[1, 16, 17] but not those reported by others.[3, 18-20] Others have noted comparable findings in hay fever and other nonasthmatic allergic disorders[17] and have been unable to distinguish mild from severe asthma on the basis of urinary steroid excretion.[18]

The method utilized for determining corticosteroid excretion measures tetrahydro-cortisone, tetrahydro-cortisol, 11-keto-androsterone, 11-β-hydroxy-androsterone, 11-keto-etiocholanolone and 11-β-hydroxy-etiocholanolone. Adrenosterone is not measured as such, but the majority of its urinary metabolites would be measured as "triones."[14] Corticosterone and its metabolites, which would not be measured with the procedure utilized, are excreted only as traces in man.[15]

It is to be emphasized that none of the asthmatic, hay fever, or control population had any clinical evidence of hypogonadism or other endocrine disorders. Furthermore, the weight and height of the asthmatic population did not appear significantly different from the nonasthmatic control subjects and hay fever patients.

Differences in methods may acount in part for the discrepancies between the results of our studies of total 11-oxysteroid excretion and those reported by Venning and colleagues,[1, 4] who bio-assayed glucocorticoid activity in the urine of asthmatic patients, and Forssell and associates,[3] who noted insulin sensitivity and hypoglycemic unresponsiveness. It is of some interest that at least two previous investigations have shown normal to above normal 17-ketosteroid excretion in asthmatic women over 45 years of age.[18, 19]

Further investigation appears to be necessary to determine the significance of reduced 17-ketosteroid excretion in relation to the etiology of bronchial asthma. Since none of the patients with mild asthma had ever received steroid medication, the endocrine changes noted cannot have been a consequence of prior therapy. It is most likely that the reduction in 17-ketosteroid excretion is an associated abnormality, similar to the reduced 17-ketosteroid excretion noted in many other types of chronic illness.[21] The fact that both male and female patients with asthma excrete smaller amounts of 17-ketosteroids than controls suggests that the adrenal cortex is primarily responsible. If this is so, the relatively normal total 11-oxysteroid excretion in persons with bronchial asthma found

in this study, as well as the normal adrenocortical responsiveness to ACTH in this disease noted by ourselves and others,[22] suggests an altered balance in the secretion of C19 and C21 steroids by the adrenal cortex. This is in keeping with the observations of Selye and others concerning the effect of chronic stress upon 17-ketosteroid and 11-oxysteroid excretion.[23] The observed failure of corticoid excretion to vary significantly with variations in severity of obstructive pulmonary disease is in accord with observations of Bliss and co-workers,[24] who noted normal blood and urine corticoid concentration in psychotic and nervously stressed nonpsychotic patients.

SUMMARY

A controlled study of 17-ketosteroid and 11-oxysteroid excretion in ambulatory patients with bronchial asthma has been carried out during intervals when the patients had been without steroid medication for at least two weeks. No significant correlation was noted between steroid excretion and the asthmatic state as verified by serial timed vital capacities and daily score sheets. No significant correlation was noted between 17-ketosteroid excretion and 11-oxysteroid excretion. Total 11-oxysteroid excretion was not abnormal in bronchial asthma. Significant reduction of total 17-ketosteroids was found in both male and female asthmatic patients, as compared to age- and sex-matched controls, but not in patients with hay fever. Fractional 17-ketosteroid excretion as measured by androsterone-glucuronide and etiocholanolone-glucuronide analyses tended to confirm total 17-ketosteroid excretion. The administration of cortisone resulted in a further decrease of androsterone-glucuronide and etiocholanolone-glucuronide excretion for the duration of therapy.

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EXPERIMENTAL TOXIC AND ALLERGIC CONTACT DERMATITIS

I. A Chemical Study of Histamine Content

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SOME pharmacologically active substances which are normal tissue constituents and are liberated at the site of an antigen-antibody reaction are today considered to be essential to certain manifestations of the *immediate* type of sensitivity. There seems to be little known about the chemical substances which occur in the lesions of primary (toxic) inflammation and of allergic inflammation of the *delayed* type of sensitivity.

The present study of the histamine content (Part I) and histopathology (Part II) of the primary (toxic) inflammation and the allergic inflammation of guinea pig skin was undertaken not only to learn about the chemical and histologic changes occurring but also, through correlated experiments, to throw some light on the source and role of histamine in the lesions induced.

In a recent study, Inderbitzin[1] reported a rise of 400 per cent above normal in allergic dermatitis and 700 per cent in a tuberculin reaction forty-eight hours after the lesion was induced. In subsequent studies the same author[2, 3] indicates that the infiltrating mononuclear cells are the possible source of histamine because of their involvement in allergic reaction. The studies cited do not record histamine measurements at the sites of a nonallergic lesion known to cause an inflammatory infiltration. This control study is important, since the white blood cells contain large amounts of histamine.[4] Further, Inderbitzin used the biologic assay with atropinized guinea pig ileum for quantitative determination of histamine. Traces of serotonin, a potent smooth-muscle activator,[5] might have been present. These two factors could have given rise to an erroneous interpretation of the results.

In the present study the allergic inflammation is represented by allergic dermatitis of the guinea pigs made sensitive to contact with a simple chemical. The primary inflammation is represented by toxic dermatitis induced in normal animals by the same but a more concentrated chemical.

MATERIALS AND METHODS

Reagents.—The following reagents were used in the study:

1-Chloro-2,4-dinitrobenzene (DNCB) (Eastman Organic Chemicals, Inc.), recrystallized from hot mixture of benzol and absolute alcohol.

From the Institute of Allergy, Roosevelt Hospital.

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1-Fluoro-2,4-dinitrobenzene (Eastman Organic Chemicals, Inc.).

Acetone (analytical grade), absolute alcohol USP, chloroform USP (Mallinckrodt Chemical Works), and olive oil USP were used as solvents.

Compound 48/80, a histamine liberator, was donated by the Burroughs Wellcome Research Laboratories, Tuckahoe, New York.

Germanin (Naphuride sodium, brand of suramin sodium), produced by Winthrop-Stearns, Inc.

l-Histidine dihydrochloride (Mann Research Lab.).

Reagents for histamine measurements as specified by Lowry and associates[4] were used.

Acid-washed and ignited sea sand (Fisher Scientific Co.).

Preparation of Skin Proteins.—Shaved skin strips, excised from the back of exsanguinated guinea pigs, of about 500 to 1,000 mg. wet weight, after the platysma had been stripped off, were frozen at −20° C. The frozen skin was cut into minute fragments into a mortar, mixed with sand, and crushed into a doughy mass with a porcelain pestle; then, upon addition of a small amount of 0.283 per cent saline (threefold dilution of 0.85 per cent saline), it was ground to a paste. This mixture of skin proteins and sand was then transferred into a Pyrex centrifuge tube of 40 ml. capacity and further ground against the walls of the tube by means of a Pyrex rod, with a cone-shaped blade, fixed in a mechanical stirrer. During the process of grinding and stirring, small portions of 0.283 per cent saline were added to a total volume of 6 to 9 ml. Several yields were mixed and stored for twenty-four hours in the refrigerator. Thereafter the stirring process was repeated and the suspension was centrifuged for one hour at 3,000 r.p.m. A sample of the supernatant was removed for protein determination; it was found to contain 0.2 to 0.4 per cent alcohol-precipitable protein. The opalescent supernatant was measured in a volumetric cylinder, freeze-dried, and stored at −4° C.

Preparation of Dinitrophenyl Conjugate.—For preparation of dinitrophenolized skin protein conjugate (DNP conjugate), the procedure followed was essentially that described by Landsteiner and Chase.[6] However, since parenteral injection of certain chemicals, without conjugation to proteins, together with tubercle bacilli also renders guinea pigs highly sensitive to contact with the chemical,[7] no effort was made to follow completely the specific instructions for obtaining soluble protein conjugate free of the chemical.

The skin proteins freeze-dried from 0.283 per cent saline were weighed and dissolved in distilled water in such amount as to establish physiologic salinity (0.85 per cent NaCl). Thirty-milliliter portions of the skin protein solution suspension were brought to alkalinity (pH 9.0) by addition of normal Na_2CO_3, and the mixture was cooled to −4° C. Upon addition of 5 ml. chloroform with 186 mg. 1-fluoro-2,4-dinitrobenzene at −4° C., the mixture was activated by shaking in a refrigerated chamber for thirty minutes and centrifuged. The chloroform layer and the sediment were discarded, and the supernatant was acidified with normal hydrochloride to a maximum precipitation of the now dinitrophenolized proteins. After collection by sedimentation, separate portions of the precipitate were suspended in a small amount of saline and, by cautious addition of normal NaOH and saline to pH of 7.0, the suspension was maneuvered into maximum dissolution in a minimal volume of saline. Some of the precipitated proteins remained undissolved. A few glass beads were added. Gentle shaking yielded a fine suspension. To establish the protein content of the suspension, a sample was precipitated with an excess of absolute alcohol and the precipitated proteins were dried and weighed. The dinitrophenolized protein solution suspension was adjusted to contain 10 mg. protein per milliliter (one per cent) by addition of 0.85 per cent saline.

Preparation of Sensitizing Mixtures of Tubercle Bacilli and Dinitrophenyl Conjugate in Adjuvant or in Saline.—Tubercle bacilli of human type (TBB) grown on a synthetic medium, washed, autoclaved, and lyophilized, were supplied by Dr. Jules Freund, Department of Health, New York City. Clumps of the bacillary mass were crushed gently in a mortar in dry state under sterile conditions. Three grams of the powdered bacilli were suspended in 100 ml. of

0.85 per cent sterile saline. Upon addition of a large number of small glass beads, the suspension was shaken at the rate of 60 oscillations per minute for forty-eight hours in a glass-stoppered Pyrex bottle (1,000 ml. capacity). The yield—a milky brown suspension of 3 per cent TBB in saline divided in several portions—was stored at –20° C.

Freund's adjuvants[8] (Bacto Adjuvant incomplete, Difco Laboratories) containing 1.5 ml. Arlacel A (mannide mono-oleate) and 8.5 ml. Bayol (paraffin oil) per 10 ml. in sterile vials, was used for preparation of emulsions of TBB with the DNP conjugate.

Shortly before injection 10 ml. portions of sensitizing emulsions were prepared by means of mechanical stirring of 1 ml. of TBB suspension (30 mg.), 1 ml. of DNP conjugate (10 mg.) and 8 ml. of Freund's adjuvant. Sterile glass beads were added to maintain the emulsification in the periods between injections by means of manual shaking. This emulsion was used for intramuscular injections only.

For intraperitoneal injections 10 ml. portions of saline suspension were prepared, each containing 60 mg. TBB and 30 mg. DNP-conjugate.

Determination of Toxicity of DNCB in Olive Oil to Skin of Normal Guinea Pigs.—Six concentrations of DNCB in olive oil (2 per cent, 1.5 per cent, 1 per cent, 0.75 per cent, 0.5 per cent, and 0.1 per cent) were used to determine the capacity of DNCB in olive oil to induce a primary inflammatory reaction (that is, primary toxic dermatitis) in the skin of normal guinea pigs.

The 2 per cent and 1 per cent solutions were prepared by weighing the DNCB and dissolving the crystals in olive oil in volumetric flasks. The remaining solutions were prepared by dilution using volumetric pipettes.

One hundred normal male albino guinea pigs were used. Groups of twenty animals were tested at one time. Two bilateral areas on the posterior two-thirds of the back (3 by 12 cm.) and one bilateral area over the nuchal region (3 by 3 cm.) were shaved by means of an electric clipper with a No. 0000 blade. No chemical dipilatory was used. Eighteen hours later the test dilutions were applied—three of the six DNCB dilutions on each of the shaved strips, with the highest concentrations to the rear. For control, plain olive oil was applied in the shorn area over the nuchal region. One drop (0.025 ml.) of each test solution was applied per site from capillary pipettes and gently spread with a glass rod over a skin area of 1.5 to 2 cm. in diameter.

TABLE I. RESULTS OF DETERMINATION OF TOXICITY OF DNCB IN OLIVE OIL TO SKIN OF NORMAL GUINEA PIGS*

ANTIGEN APPLIED EPICUTANEOUSLY	REACTION		
	TYPE OF REACTION	NUMBER	PER CENT
Olive oil	Irritation	5	5
0.1% DNCB in olive oil	Toxic dermatitis	0	0
0.5% DNCB in olive oil	Toxic dermatitis	1	1.05
0.75% DNCB in olive oil	Toxic dermatitis	3	3.15
1.0% DNCB in olive oil	Toxic dermatitis	10	10.5
1.5% DNCB in olive oil	Toxic dermatitis	79	83.0
2.0% DNCB in olive oil	Toxic dermatitis	95	100.0

*One hundred normal albino guinea pigs.

During the testing the animals were placed in small cages which restrained their movements, and boots made of adhesive tape were applied to the claws.

Readings of the tested sites were made six, twenty-four, forty-eight, and seventy-two hours after application of the test solution. If no macroscopic changes occurred within seventy-two hours the test was considered negative. Any macroscopic change (erythema, induration, or epidermal damage) was regarded as evidence of an inflammatory response induced by DNCB and recorded as primary toxic dermatitis. Five per cent of the animals showed slight irritation at the site treated with plain olive oil and the tests made on them with DNCB solutions were not recorded. Table I shows that a 2 per cent solution of DNCB in olive oil

is a toxic concentration, since it induced a primary inflammation (toxic dermatitis) in 100 per cent of the animals. The 0.1 per cent solution did not produce any macroscopic change in normal guinea pigs. This observation is supported by the study of Nilzen,[9] who did not induce any reaction to 0.1 per cent DNCB in olive oil in all of 120 guinea pigs, even though they had epicutaneous contact with DNCB twelve days before.

Therefore, 2 per cent DNCB or higher concentrations were used to induce toxic dermatitis of the skin of normal animals, whereas 0.1 per cent DNCB or less was used as a challenge for the induction of allergic reaction in sensitized animals.

Sensitization of Experimental Guinea Pigs.—The procedure which rendered guinea pigs highly sensitive to contact with the simple chemical is largely the double-step method described by Chase.[10, 11] It deviates from his method (1) in the use of animals which had prior contact with the chemical, (2) in the stimulation of the skin by epicutaneous contact with the chemical simultaneously with the intramuscular injection of the sensitizing emulsion, and (3) in the use of guinea pig skin protein conjugate instead of erythrocyte conjugate.

The guinea pigs used to determine the toxicity of DNCB were given an intraperitoneal injection of 6 mg. TBB and 3 mg. DNP conjugate in 1 ml. saline seventy-two hours after the epicutaneous testing. Seven days later two intramuscular injections, each containing 1.5 mg. TBB and 0.5 mg. DNP conjugate in 0.1 ml. saline emulsified with 0.4 ml. adjuvant, were made, one in each thigh. Simultaneously, a drop of 2 per cent DNCB in acetone was applied to the freshly shaved bilateral sites in the posterior region of the extreme lateral region of the abdomen. Seven days later the procedure was repeated by giving two intramuscular injections, this time one in each pectoral muscle, each containing 0.75 mg. TBB and 0.25 mg. DNP conjugate in 0.05 ml. saline emulsified with 0.2 ml. of adjuvant. Simultaneously, a drop of 1 per cent DNCB in acetone was applied to the freshly shaved bilateral sites anterior to those previously used. For three weeks thereafter, weekly bilateral epicutaneous applications of a drop of 1 per cent DNCB in acetone were made at fresh sites anterior to those last used. Since olive oil had proved irritating to the skin of some guinea pigs, acetone was used instead as the solvent for DNCB for epicutaneous sensitization.

Determination of Sensitivity of Contact Type.—Testing for reactivity of the sensitized animals was done four weeks after the last epicutaneous application of DNCB in acetone. From the stock of sensitized guinea pigs, seventy-five animals comparable in physical appearance, muscular tonus, and weight (450 to 500 grams) were selected for the tests.

Five concentrations of DNCB in olive oil (0.5 per cent, 0.1 per cent, 0.01 per cent, 0.001 per cent, and 0.0005 per cent) and plain olive oil were applied on two bilateral strips (3 by 12 cm.) of skin on the posterior two-thirds of the back. Eighteen hours before, the sites had been shaved with an electric hair clipper. The 0.5 per cent, 0.1 per cent, and 0.01 per cent solutions were prepared by weighing the DNCB and dissolving the crystals in olive oil in volumetric flasks; the 0.001 per cent and 0.0005 per cent solutions were obtained by dilution by means of volumetric pipettes. Three test solutions were applied on each side, the highest concentration to the rear. One drop (0.025 ml.) of each test solution was applied from a capillary pipette and gently spread with a glass rod to cover a skin area of about 1.5 to 2 cm. in diameter. During the testing period the animals were restrained as already described.

Readings of the tested sites were made six, twenty-four, forty-eight, and seventy-two hours after application of the test solution. If no macroscopic change occurred within seventy-two hours, the test was considered negative. Erythema, with or without an induration, was regarded as evidence of an allergic response and was so recorded. Three of the seventy-five tested animals showed slight irritation at the sites tested with plain olive oil and were excluded; the tests made on them with DNCB solutions were not recorded.

The results in Table II show that a high degree of sensitivity of the contact type had been induced, inasmuch as 83 per cent of the animals reacted to 0.1 per cent DNCB and 28 per cent reacted to 0.001 per cent DNCB. This method of testing established the varying degrees of sensitivity as gauged by the concentration of antigen necessary to induce an allergic reaction.

TABLE II. RESULTS OF DETERMINATION OF THE DEGREE OF CONTACT TYPE SENSITIVITY AS GAUGED BY THE CONCENTRATION OF ANTIGEN NEEDED TO INDUCE ALLERGIC REACTION*

ANTIGEN APPLIED EPICUTANEOUSLY	REACTION		
	TYPE OF REACTION	NUMBER	PER CENT
Olive oil	Irritation	3	4
0.0005% DNCB in olive oil	Allergic dermatitis	7	9.7
0.001% DNCB in olive oil	Allergic dermatitis	20	27.7
0.01% DNCB in olive oil	Allergic dermatitis	39	54.1
0.1% DNCB in olive oil	Allergic dermatitis	60	83.3
0.5% DNCB in olive oil	Allergic dermatitis	72	100.0

*Seventy-five male albino guinea pigs sensitized to DNCB.

For the purpose of the study, it was thought best to use animals with reactions that were comparable in severity. Therefore, seventy of the guinea pigs were retested twenty-one days later with 0.1 per cent DNCB in olive oil only. The criteria for the gradings were the intensity of the erythema and the thickness of the induration of the lesions. A drop (0.025 ml.) of 0.1 per cent DNCB in olive oil was applied to the skin of the nuchal region. The testing techniques detailed above were followed. The reaction was examined twelve, twenty-four, and forty-eight hours later. The intensity of the reactions is represented by the following gradings: (+++) bright red, with marked thickening of the lesion due to induration of the dermis; (++) pink-red, with distinct induration of the dermis; (+) pink, with thickening of the dermis.

TABLE III. RESULT OF DETERMINATION OF THE DEGREE OF CONTACT TYPE SENSITIVITY BY COMPARISON OF SEVERITY OF AN ALLERGIC REACTION*

TEST SUBSTANCE (APPLIED EPICUTANEOUSLY)	ALLERGIC DERMATITIS REACTION		
	DEGREE OF CUTANEOUS REACTION	NUMBER	PER CENT
0.1% DNCB in olive oil	+++	31	44.3
	++	13	18.6
	+	17	24.3
	Negative	9	12.8
	Totals	70	100.0
Control test with olive oil	Negative	70	100.0

*Seventy male albino guinea pigs sensitized to DNCB.

The results of the test appear in Table III. The forty-four animals reacting with +++ and ++ reactions were used in this study of histamine content and histopathology of induced allergic contact dermatitis.

Skin Biopsies for Histamine Measurement and Histologic Examination.—One drop of DNCB solution was spread on the shaved skin over the nuchal region to induce toxic dermatitis or allergic dermatitis. The same technique was used in control experiments with plain olive oil. A drop (0.05 ml.) of test solution, spread with a glass rod, covered an area of skin about 3 cm. in diameter, approximating 1 to 2 grams of skin. The site had been closely shaved eighteen hours before. Lifting of the skin outside the reacting area produced a fold running through the dermatitis lesion; a biopsy was taken with sharp scissors causing minimal injury to the specimen. The technique of obtaining biopsies with a punch key was abandoned after preliminary trials. It was noted that resultant vascular injuries caused hemorrhages and qualitative changes in the infiltrate. The skin specimen was blotted free of blood and cut in half; one-half of the specimen was immersed in formalin, to be processed later for histologic examination. For histamine measurement, the other half was carefully cleansed with a cotton swab and ether of remnants of the DNCB in olive oil, then freed of platysma, hair stumps, and scales, frozen at –20° C., weighed on an analytical balance, and kept in a deep freeze.

Only one lesion was induced in a guinea pig at a time, and one biopsy specimen was removed. However, for the investigation of the effect of germanin and l-histidine on the histamine content in the allergic lesions, two groups of guinea pigs were re-used after a fifteen-week interval.

Histamine Measurement.—For quantitative determination of histamine in the skin, in the whole blood, and in the lymph node tissue, an adaptation of the microchemical method of Lowry and associates[1] was used. This method consists of eliminating most of the interfering substances in tissue extracts, concentrating the histamine by adsorption on Decalso columns. Then follows the formation of the basic monodinitrofluorobenzene derivative of histamine and extraction of this derivative into methyl-n-hexyl ketone and out again into hydrochloric acid. The optical density of the acid solution is measured at the 360 mμ wave length on a Beckman spectrophotometer in quartz microcuvettes.

Lowry and colleagues have shown that this method is sensitive and specific when applied to buffy coats and platelets of human origin.[12] However, the amount of "determined" histamine in human plasma, erythrocytes, and whole blood is increased by interfering substances not wholly eliminated. Spermine, spermidine, putrescine, cadaverine, histidinol, and agmatine may cause higher readings. There is very little histamine present in the erythrocytes, and the "determined" histamine content may be almost all attributed to spermine and spermidine. The red cells contribute most of the interfering material present in the whole blood. The authors estimated that the "determined" amount of histamine in the whole blood represents a value approximately 20 per cent higher than the actual amount of histamine which is carried largely by the leukocytes.

Johnson[13, 14] recently published an adaptation of the above chemical measurement of histamine in the skin which employed homogenization of the specimens and extraction of the histamine without acid hydrolysis of the protein.

A similar technique used in the present study was as follows: The skin specimens frozen at −20° C. were cut into minute fragments into a porcelain mortar and mixed with a measured amount of sand. The mixture of skin and sand was crushed with a porcelain pestle; the doughy mass was then transferred to a Pyrex centrifuge tube. One milliliter of 0.1 per cent histamine-releasing Compound 48/80 in 0.85 per cent saline was first used to rinse the walls of the mortar and then added to the mixture of crushed skin and sand in the centrifuge tube. There the pasty mass was ground for five minutes by means of a Pyrex glass rod fixed in a mechanical stirrer. Thirty minutes later 3 ml. of 5 per cent trichloroacetic acid (TCA) was added and the grinding was repeated. Then 2 ml. of 30 per cent TCA were added, increasing the concentration of TCA in the mixture to 12.5 per cent. The tubes were stored overnight at −4° C. The following morning the tubes were centrifuged for fifteen minutes at 3,000 r.p.m., and an aliquot of the supernatant was used for histamine determination.

Preliminary experiments had shown that extracts prepared with 5 per cent TCA (pH 1.1) were not sufficiently deproteinized. The protein adsorbed on the Decalso plugged the columns. The use of 12.5 per cent TCA (pH 0.9) resolved the difficulty and did not adversely affect the readings; nor did overnight storage of the extracts at −4° C. have an adverse effect on the readings.

As stated, the chemical method of histamine measurement has a definite, though limited, error when applied to whole blood, erythrocytes, or plasma and, therefore, might have an error when applied to other tissues. This error was minimized by the use of identical techniques, and it becomes less significant since the main objective of this study was not so much to measure the absolute values as to contrast histamine changes in corresponding tissues.

All histamine values reported in this study are expressed in micrograms of histamine base.

RESULTS AND DISCUSSION

Normal.—Fig. 1 shows the histamine content of the normal skin in micrograms per gram of skin following one epicutaneous application of plain olive

oil. The histamine content of the skin remained within the normal limits (2 to 6 mcg., average 4.3 mcg.) for the period of six days, during which time these determinations were made.

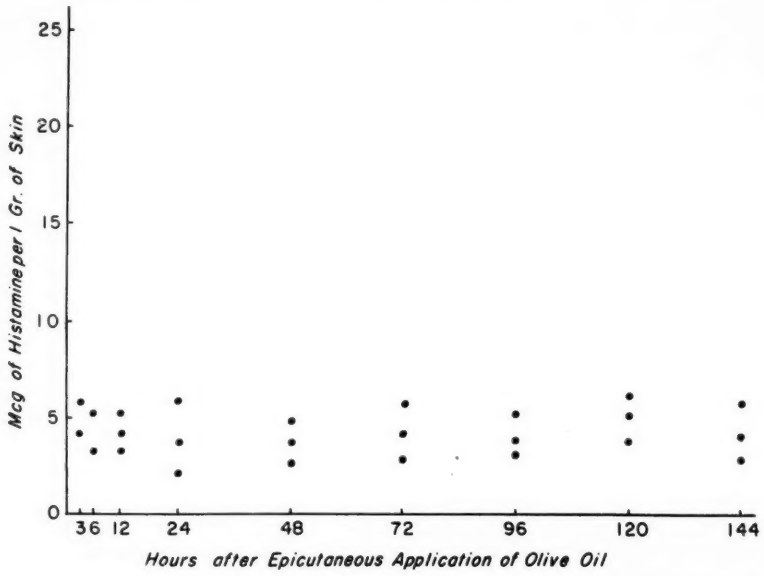

Fig. 1.—Histamine of normal guinea pig skin after epicutaneous application of olive oil. Each dot represents the determined value of histamine base in one animal.

Allergic.—Fig. 2 presents the histamine content in biopsies taken at the sites of allergic dermatitis of sensitized guinea pigs after a single epicutaneous application of 0.1 per cent DNCB, a nontoxic concentration, in olive oil. A steady rise in histamine begins after the challenge, and the maximum is reached at forty-eight hours. At this stage in the development of the allergic lesion the histamine has risen to an average of 20.2 mcg., which is more than 400 per cent above the average amount measured in normal skin at the sites of olive oil controls. Thereafter, on the third, fourth, and fifth days, the histamine rapidly diminishes and the allergic dermatitis quickly regresses. On the fifth day very little erythema is left and the lesion is covered with a cigarette-paper-thin scale. It should be noted that the macroscopic changes at the site of allergic dermatitis seem to reach their maximum twelve to twenty-four hours after the beginning of the reaction, thus preceding the histamine peak by more than twenty-four hours. The number of infiltrating cells, as seen in the histologic section, also reaches the maximum in the lesions of twelve to twenty-four hours' duration and remains undiminished through the second, third, and fourth days.

Toxic.—The histamine content at the sites of toxic dermatitis induced in normal guinea pig skin by application of 2 per cent and 5 per cent DNCB in olive oil is shown in Fig. 3. A single application of 2 per cent DNCB to skin

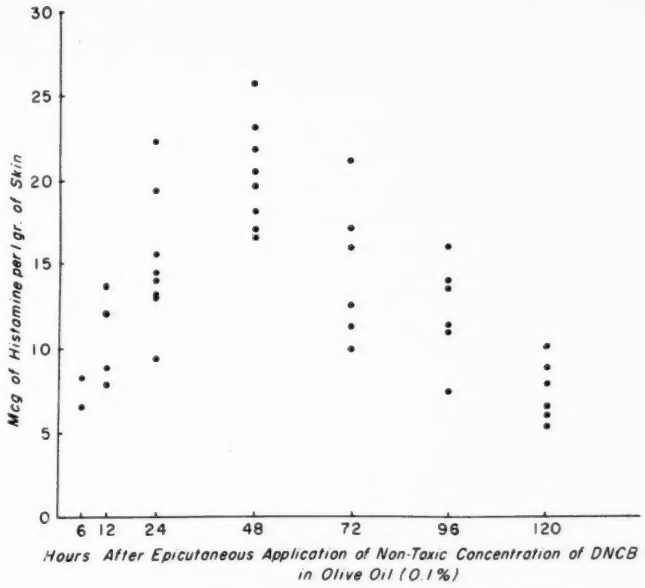

Fig. 2.—Histamine at sites of allergic DNCB dermatitis of sensitized guinea pigs. Each dot represents the determined value of histamine base in one animal.

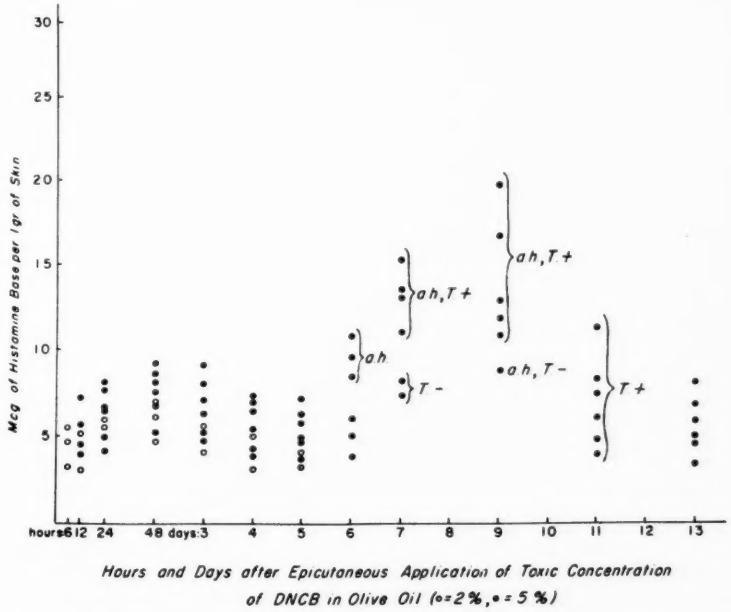

Fig. 3.—Histamine at sites of toxic DNCB dermatitis of normal guinea pigs. Each dot represents the determined value of histamine base in one animal.

a.h., Histologic changes indicating allergic process beneath the slough of the toxic reaction. No such changes were found in three biopsies made on the sixth day and two biopsies on the seventh day. Skin specimens obtained on the eleventh day and thirteenth day were not examined histologically.

T.+, A positive epicutaneous test with 0.75 per cent DNCB. On the seventh, ninth and eleventh days, after biopsies were made, a new area of skin of each guinea pig was shaved. One drop (0.025 ml.) of 0.75 per cent DNCB in olive oil was applied. Twenty-four hours later these sites were inspected for erythema and induration. On the sixth and the thirteenth days no epicutaneous testing was done.

T.–, A negative test with 0.75 per cent DNCB.

of normal guinea pigs induces a primary inflammation in all the animals. There were changes in the histamine content of the lesions, but the results were not significant. The differences in individual measurements overlapped and closely approximated the range of histamine content of normal skin. The next experiments were therefore made with one application of 5 per cent DNCB in olive oil. At these sites the histamine content rises and reaches a maximum in forty-eight to seventy-two hours, about 80 per cent above the amount of histamine in normal skin, in sharp contrast with the 400 per cent histamine increase at the site of the allergic lesion. On the third, fourth, and fifth days the toxic lesions regress and the histamine seems to diminish. The decrease is small and contrasts with the rapid disappearance of histamine from allergic lesions of the same duration.

A comparison of the intensity of macroscopic changes in toxic lesions with the intensity of changes in allergic lesions is not warranted. The microscopic comparison of these inflammatory processes shows that the infiltrating cells in allergic dermatitis are mononuclear cells, whereas in toxic dermatitis they are both polymorphonuclears and mononuclears in about the same proportion as found in blood. The number of cells in the allergic lesions induced by 0.1 per cent DNCB and in toxic lesions induced by 5 per cent DNCB are essentially the same. However, the cell infiltration in an allergic lesion reaches the maximum in twelve to twenty-four hours, whereas in a toxic lesion the infiltration takes place at a slower pace (maximum of forty-eight to seventy-two hours). Yet, and this is noteworthy, the histamine maximum in the toxic lesion is simultaneous with the maximum infiltration, whereas the maximal histamine rise in the allergic lesion is not attained until twenty-four hours after the cellular infiltration has reached its peak. These differences indicate a basic dissimilarity in the mechanisms of histamine accumulation in toxic and allergic dermatitis.

Some of the toxic lesions begin to flare up on the sixth day. The histamine mounts and reaches a new peak about the ninth day, over 300 per cent above the histamine content of normal skin. The histologic changes in the regenerated epidermis underneath the slough of the toxic reaction have, in some biopsies, the histologic characteristics of allergic contact dermatitis (*a.h.* in Fig. 3). A new infiltrate of mononuclears appears, first mainly perivascular (sixth and seventh days), then diffuse between collagen fibrils (ninth day). Foci of the mononuclear invasion of the regenerated epidermis, resembling allergic vesicles, were found in all specimens. Epicutaneous testing with 0.75 per cent DNCB in olive oil on a new area of the skin revealed a development of contact sensitivity in some animals by the seventh day and in all animals by the eleventh day. The second histamine rise and the histologic changes in the biopsies coincide with the acquisition of general allergic sensitivity. It is conceivable that antigen left in the slough or combined with the living tissues induced an allergic flare-up at the site of the primary lesion accounting for the histamine accumulation and specific histologic changes described in detail in the subsequent article.

A control experiment was set up to confirm this assumption. First, an epicutaneous application of 5 per cent DNCB in olive oil was made, inducing toxic dermatitis in ten normal guinea pigs. Eight days later applications of 0.75 per cent DNCB in olive oil and of plain olive oil were made to freshly shaved sites. The next day five of the ten guinea pigs showed a positive reaction at sites challenged with 0.75 per cent DNCB. These five animals were sacrificed, and biopsies for histamine measurement were obtained from (1) the original toxic lesions now secondarily flaring, (2) the allergic reactions resulting from the challenge with 0.75 per cent DNCB, and (3) the control sites tested with plain olive oil.

Skin sites tested with plain olive oil had normal levels of histamine. The fresh twenty-four-hour allergic lesions, as well as the nine-day-old toxic lesions, contained about 300 per cent more histamine than the normal skin, just as those nine-day-old lesions shown in Fig. 3.

Histamine determinations at the sites of allergic dermatitis, at the sites of toxic dermatitis, and in the control experiment just described demonstrate that a higher level of histamine is specifically associated with allergic response (contact dermatitis) but not with primary inflammatory response (toxic dermatitis) in normal animals prior to development of sensitization.

In Fig. 4 is presented a summation of data herein reported, as well as some additional information to facilitate a discussion of the probable source of the histamine accumulating at the sites of primary toxic and allergic inflammation.

In the first and second columns are contrasted the histamine content of the whole blood and of the skin of sensitized guinea pigs as opposed to normal guinea pigs. Obviously, there is no essential difference in the histamine content.

In the third column are presented the histamine measurements at the sites of toxic dermatitis of forty-eight hours' duration induced by 2 per cent, 5 per cent, and 10 per cent DNCB in olive oil and those induced by 1 per cent, 5 per cent, and 10 per cent DNCB in acetone in normal animals. The difference between the two groups is insignificant. The solvent of DNCB influences the accumulation of histamine very little.

The increase in the concentration of DNCB does intensify the inflammatory reaction, with a simultaneous increase in the infiltrating cells. Since the white blood cells contain most of the histamine determined in whole blood, the quantitative increase of cellular infiltration results in a higher level of histamine. This is shown by comparing the histamine levels in lesions induced by 2 per cent DNCB with those induced by 5 per cent DNCB in olive oil, and in lesions induced by 1 per cent DNCB with those induced by 5 per cent DNCB in acetone. In lesions of toxic dermatitis induced by 10 per cent DNCB, however, less histamine is found than in lesions induced by 5 per cent DNCB. A comparison of macroscopic and microscopic findings revealed the following: Within three to six hours after application of 1 per cent, 2 per cent, or 5 per

cent DNCB, the skin becomes progressively erythematous and indurated, reaching a maximum in forty-eight to seventy-two hours. The toxic damage involves mainly the epidermis and a very thin layer of the uppermost portion of the dermis. In contrast to this, the skin tested with 10 per cent DNCB flares up within a few hours, but for a very short time. Thereafter, the lesion becomes progressively ischemic. The toxic damage extends into the deepest layer of the dermis, involving the deep blood vessels. This vascular damage is manifested by thrombotic occlusion. The infiltrating cells cannot reach the lesion. Around the forty-eight-hour lesion there is a narrow erythematous zone. The biopsies were made from the central ischemic portion. The aforementioned assumption that the histamine content of the toxic erythematous inflammation depends mainly on the number of infiltrating cells is supported by the fact that the histamine level is low in the toxic ischemic inflammation where these cells are absent.

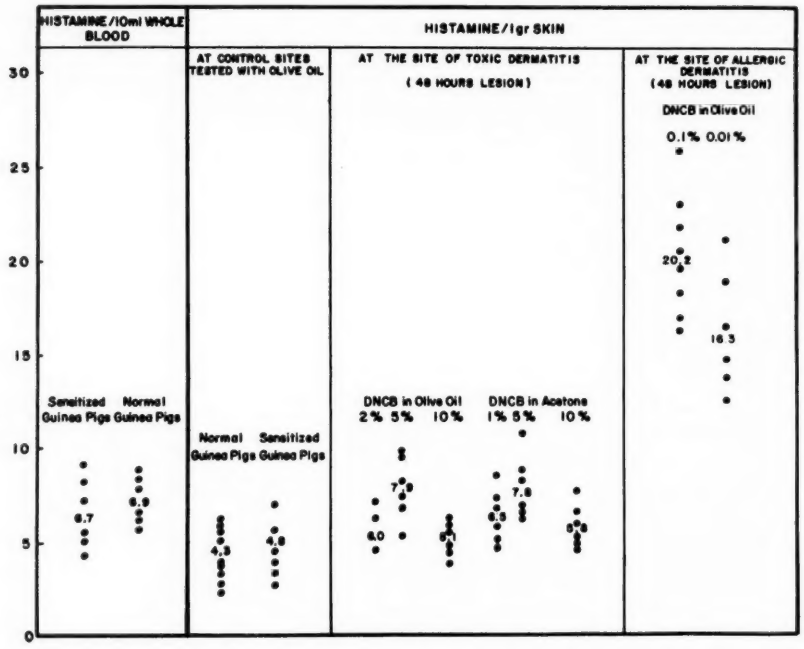

Fig. 4.—Comparative data concerning histamine levels in whole blood, in normal skin, and at the sites of toxic and allergic dermatitis.

In the fourth column of Fig. 4 are contrasted the histamine levels at the sites of allergic dermatitis at forty-eight hours induced by 0.1 per cent and 0.01 per cent DNCB in olive oil. The tenfold reduction in the concentration of DNCB decreased the histamine content and the number of infiltrating mononuclear cells of the allergic lesions by almost one-third. The number of cells in the allergic lesions induced by 0.01 per cent DNCB is about the same as in toxic lesions induced by 2 per cent DNCB and more than one-third less

than in those induced by 5 per cent DNCB. Nonetheless, the average histamine content (16.3 mcg.) of these allergic reactions (0.01 per cent DNCB) is more than twice that (7.9 mcg.) measured at the sites of toxic dermatitis (5 per cent DNCB).

Subtracting the average amount of histamine at the control sites from the average amount of histamine in the forty-eight hour lesions of allergic dermatitis yields an average histamine increase in the allergic lesions. This amounts to 15.4 mcg. of histamine in lesions induced by 0.1 per cent and 11.5 mcg. in those induced by 0.01 per cent DNCB. If the infiltrating white blood cells should be solely responsible for the import of histamine into the site of allergic dermatitis, as is assumed for toxic dermatitis, it would require all the leukocytes from 19 to 25 ml. of blood—more blood than a 500 gram guinea pig has. White blood cell counts and differential blood counts were made before, during, and after the allergic reaction without revealing any significant changes; furthermore, there were no differences in the histamine content of the whole blood taken at the same time. In the blood of 500 gram guinea pigs, 10,000 to 20,000 leukocytes were counted in 1 cmm., differentiated into 20 to 40 per cent polymorphonuclears, 60 to 80 per cent mononuclears, 1 to 8 per cent eosinophils, and 0 to 1 per cent basophils.

In the skin of highly sensitive guinea pigs, 3 per cent concentration of DNCB induces ischemic reactions comparable in macroscopic appearance to those induced in normal animals by 10 per cent DNCB. The histamine within the ischemic lesions of sensitive guinea pigs (48 and 72 hours) did not exceed the histamine content of the toxic reactions induced in normal animals. Evidently, the skin tissue of highly sensitive animals is not capable of histamine accumulation after the infiltrative process is obstructed. This leads to the assumption that the infiltrating mononuclear cells play an important role in the excessive histamine accumulation at the site of allergic dermatitis.

As stated previously, the number of cells infiltrating the allergic lesions induced by 0.01 per cent DNCB is smaller than that in the toxic lesions induced by 5 per cent DNCB; nonetheless, the histamine level is double that of toxic reactions. Furthermore, there is no significant difference in the number of infiltrating cells at the site of either allergic dermatitis induced by 0.1 per cent DNCB and toxic dermatitis induced by 5 per cent DNCB, although a marked difference in histamine levels was demonstrated. The leukocytes in normal and in sensitive animals contain the same amount of preformed histamine, and no significant changes in histamine content of whole blood were observed during or after an allergic reaction. The qualitative cellular difference in the infiltrate at the sites of toxic versus allergic dermatitis seem too small to account for the marked difference in histamine levels in the two different inflammatory reactions. Thus, it may be assumed that the infiltrating mononuclear cells of sensitized animals have the capacity, upon minute specific stimulation with an antigen, to activate histamine metabolism at the site of an allergic inflammation by promoting histamine synthesis, or by inhibiting the histamine breakdown, or by both.

To support the assumption that the histamine accumulation in allergic dermatitis is brought on by alteration of histamine metabolism, two experiments were made.*

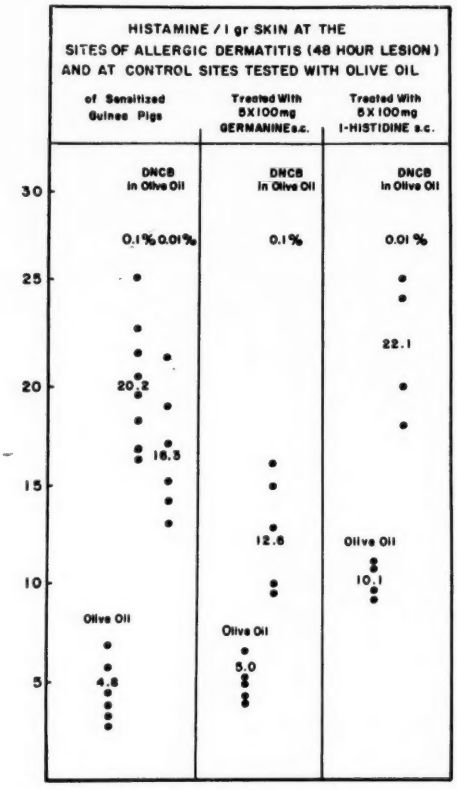

Fig. 5.—Comparative data concerning histamine levels in allergic dermatitis of guinea pigs injected with germanin and l-histidine.

The results are shown in Fig. 5. The first column presents the histamine levels at sites of allergic dermatitis induced with 0.1 and 0.01 per cent DNCB in olive oil. Fifteen weeks later the group challenged with 0.1 per cent DNCB was injected with germanin, and the group challenged with 0.01 per cent DNCB was injected with l-histidine. While being injected, each group was retested with the original challenging dose. In the second and third columns of Fig. 5 are recorded the histamine measurements in the allergic lesions induced in these two groups. There is much less histamine in the allergic lesions

*Following an allergic reaction, the skin has been found depleted of histidine, the metabolic precursor of histamine.[15] Administration of l-histidine to guinea pigs results in an increase of tissue histamine.[16-18] Experiments with radioactive histidine and histamine have shown that the tissue cells contain a mechanism for binding endogenous histamine. Exogenous histamine is excreted more rapidly than histamine formed from l-histidine in the tissue cells.[19] In vivo and in vitro experiments have shown that histamine is produced in animal tissues from l-histidine by decarboxylation reaction carried out by tissue decarboxylases.[20-24] The presence of decarboxylases has been detected in the kidney, liver, pancreas, small intestine, and skin. Hydroxylamine, semicarbazide, dinitrophenylhydrazine, brenzcatechin, and pyrogallol, among others, inhibit the decarboxylation of l-histidine and simultaneously inhibit the destruction of histamine by histaminase.[23, 24] Only germanin was reported as a decarboxylase inhibitor which had no effect on histaminase, thus preventing formation of histamine from l-histidine but not influencing the destruction of histamine.[24]

of animals injected with germanin. The histamine content increases in the allergic lesions of animals injected with l-histidine; however, a similar histamine increase was found throughout the entire integument. (Compare, in Fig. 5, the histamine content of the control biopsies of the skin tested with plain olive oil.) It would seem that germanin, an inhibitor of histamine formation from l-histidine, has interfered with the decarboxylation at the site of allergic dermatitis. This supports the assumption that the histamine accumulation in allergic dermatitis is brought on by activation of histamine metabolism. The fact that the excessive supply of l-histidine, the histamine precursor, does not promote a much greater increase of the histamine in the allergic lesion than in the integument does not necessarily weaken this assumption. It might indicate merely that the degree of decarboxylase activity is responsible for histamine increase and not the amount of l-histidine introduced into the metabolic process.

An explanation of the role of histamine in allergic dermatitis cannot be given at this time. Considering the observation that allergic lesions in guinea pigs injected with germanin were of much longer duration than allergic lesions running their course, it seems in order to suggest that histamine is an accelerator in the repair process rather than a causal factor in these allergic reactions.

SUMMARY

1. Histamine determinations at the sites of allergic dermatitis and toxic dermatitis demonstrate that excessively high levels of histamine are specifically associated with the allergic response (contact dermatitis) but not with the primary inflammatory response (toxic dermatitis).

2. The histamine increase in toxic dermatitis is attributed to the number of infiltrating cells, whereas it is assumed that the excessive histamine accumulation in allergic dermatitis is due to the capacity of the infiltrating mononuclears, upon minute stimulation with antigen, to activate the histamine metabolism. The latter assumption is supported by the result of an experiment with germanin, an inhibitor of decarboxylation of l-histidine into histamine.

3. The role of histamine in the allergic dermatitis seems to be that of an accelerator of the repair process rather than of a causal factor.

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EXPERIMENTAL TOXIC AND ALLERGIC CONTACT DERMATITIS

II. A Histopathologic Study

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With the Technical Assistance of Sonya Stroyman and Gloria Gordon

INTRODUCTION

THE early investigators of the allergic contact type of dermatitis of the guinea pig[1-5] reported and emphasized that the histologic changes in allergic contact dermatitis of guinea pigs resemble those in contact dermatitis of human beings. Later investigators[6-11] not only abstained from likening the histology of allergic contact dermatitis of the guinea pig to that of human lesions, but some also stated that morphologic and histologic changes seen regularly in human skin cannot be induced readily, if at all in the skin of guinea pigs. Of special interest, therefore, are the recent publications of Jadassohn, Bujard, and Brun,[12, 13] DeWeck and Brun,[14-16] and Baer, Rosenthal, and Sims,[17] who have revived the controversy. These investigators have demonstrated in the epidermis of the guinea pig mammilla and in the skin, after the epidermal cells have been exogenously induced to proliferation and acanthosis, histologic changes which can be compared to those observed in human epidermis at the sites of contact dermatitis.

The terms describing histologic changes seen in experimental contact dermatitis are derived from the terminology used for histopathologic description of several types of eczematous dermatitis of human skin before the specific etiology and pathogenesis of contact dermatitis were recognized and acknowledged as manifestations of induced sensitivity. An attempt has been made to fathom the concepts and interpretations in original texts by authors who suggested terms or used them for histopathologic diagnosis of eczematous dermatitis of human skin:

Alteration cavitaire (Leloir[18]): Perinuclear vacuolization of epidermal cells.

Acanthosis (Unna[19]): A thickening through hyperplasia of rete malpighii.

Spongioide umwandlung (Unna[19]), *Spongiose* (Besnier and Doyon[20]): Spongiosis through intercellular edema of the epidermis which, when marked, progresses to vesiculation.

Vesicle de Willan[21] (Brocq[22]): A circumscribed globoid space within the epidermis, filled with serous fluid usually containing a number of lymphocytes. The compression of epidermal cells surrounding the vesicle suggests its rapid development through localized intercellular pressure.

Vesicle primordialle sous-cornee (Civatte[23]): A primary vesicle located in the uppermost portion of the epidermis, beneath the keratinized surface, arising in place of disintegrating epidermal cells.

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Recently Miescher[24-26] studied chronologically the development of histologic changes occurring in human skin at sites of contact dermatitis and primary toxic dermatitis. He added a concept which alludes to a physiopathologic process in the development of contact dermatitis *(Spongiose lymphocytaire ascendent precoce en foyer)*. He demonstrated, in biopsies obtained from early lesions of contact dermatitis induced in sensitive patients by means of patch tests, evidence of focal spongiosis associated with a precocious ascent of lymphocytes through the dermis and the dilated intercellular spaces of the epidermis. Similar histologic findings are reported by Charpy, Stahl, and Castelain.[27] These authors, however, are of the opinion that the changes seen in the epidermis are only epithelial manifestations of an exudative allergic process of the papillary portion of the dermis (Manifestation epithelial de allergie exudative du derme pappillaire).

In the present study is contrasted the development of the histopathology of allergic dermatitis and of primary toxic dermatitis of the guinea pig in an effort to establish a difference, if any exists, between the physiopathologic processes of the two types of inflammatory reactions. The allergic dermatitis is represented by contact dermatitis of guinea pigs made extremely sensitive to a simple chemical (1-chloro-2,4-dinitrobenzene, referred to as DNCB). The primary dermatitis is represented by toxic dermatitis induced in normal animals by the same, but a more concentrated, chemical. It should be noted at this time that in guinea pigs sensitized to contact with DNCB and other simple chemicals, by any procedure (epicutaneous contact or parenteral injections with or without adjuvant), the delayed and the immediate types of sensitivity are always* induced simultaneously.[29-31]

MATERIALS AND METHODS

The techniques used (1) to determine the toxicity of DNCB in olive oil to skin of normal guinea pigs, (2) to sensitize guinea pigs, (3) to test the contact type of sensitivity, and (4) to obtain skin biopsies are described in detail in the first part of this study.[32] As stated there, only one lesion was induced in each guinea pig at one time, and only one biopsy specimen was removed. For a detailed examination of the histologic changes at the site of allergic dermatitis, some highly sensitive guinea pigs were used two to three times. The resting period between biopsies was at least six weeks. For a detailed examination of the histologic changes at the sites of primary toxic dermatitis, normal guinea pigs with no prior contact with the chemical were used.

Biopsy specimens from sites of allergic dermatitis were obtained three, six, nine, twelve, twenty-four, and forty-eight hours and three, four, and five days after epicutaneous challenge with nontoxic (0.1 per cent, 0.01 per cent) concentrations of DNCB in olive oil. Six to ten guinea pigs were biopsied at each interval. Biopsies for investigation of histologic changes at the sites of primary toxic dermatitis were made in the same sequence, using normal guinea pigs and toxic (2 per cent, 3 per cent, and 5 per cent) concentrations of DNCB in olive oil. In addition, biopsies obtained from lesions of six, seven, and nine days' duration, induced by 5 per cent DNCB in olive oil, were examined. At each interval four to five animals were biopsied from 2 per cent and 3 per cent DNCB lesions and six to ten from 5 per cent DNCB lesions.

Plain olive oil proved slightly irritating to the skin of 5 per cent of normal animals.[32] Furthermore, it has been reported that a daily application of plain olive oil to guinea pig skin for ten consecutive days induces an average twofold acanthosis of the epidermis.[33, 34] Therefore, a number of biopsies obtained from normal and sensitive animals from sites

*Substantiated by our unpublished experiments using passive cutaneous anaphylaxis[28] for demonstration of serum antibodies.

tested with plain olive oil were histologically examined. One application of plain olive oil did not induce any significant changes in the epidermis or dermis, even if an irritation had been observed at the time the biopsy was made or before. Occasional epinuclear vacuolization of superficial epidermal cells was seen. However, such epinuclear vacuolizations were also found in the epidermis of skin areas shorn of hair with an electric hair clipper without application of olive oil.

Preparation of Histologic Sections.—Part of each skin biopsy specimen used for measurement of histamine content[32] was examined histologically. These specimens were fixed in formalin. Additional biopsies, made to gather supplementary histologic information, were fixed in Bouin's fixative.

The fixed specimens were mounted in paraffin and secured in the microtome in such position as to yield cross sections of the hair follicles (the hair shafts pointing at the microtome blade at an angle of about 30 degrees). Twelve serial sections were made at two levels of each specimen, leaving one-half of each. After microscopic examination of these sections, the remaining half of some were cut for more thorough examination. Some were remounted and the paraffin blocks were fixed in the microtome in such position as to yield longitudinal sections of the hair follicles.

Routine Harris' hematoxylin and eosin stain was used.[35] Six serial sections of each biopsy specimen were stained with Lillie's azure A-eosin B method[35] to reveal tissue mast cells and blood basophils and eosinophils. None of these cells were found in excess.

The microphotographs of the histologic changes were made in color on Anscochrome film and then were converted into black and white negatives for printing and reproduction.

RESULTS

Histologic Changes at the Site of Allergic Contact Dermatitis.—Three hours after a nontoxic concentration (0.1 per cent and 0.01 per cent) of DNCB in olive oil has been applied to the shorn skin of highly sensitized animals, the epidermis is not affected. The main changes are in the dermis. The capillaries of the upper and middle portions of the dermis are dilated, and a slight interstitial edema is present. The infiltrating cells, exclusively mononuclears, are clinging to the vascular endothelium, many leaving the lumen (Fig. 3). A number of these cells are perivascular, and a small amount are diffuse between the collagen fibrils (Fig. 2). Some of the infiltrating cells are in a linear arrangement, giving an impression of trails ascending toward the epidermis (Figs. 1 and 2). Here a few cells which have reached the basement membrane of the epidermis or are in close proximity are escorted or surrounded by a vacuole. A formation of vacuoles adjacent to the mononuclear cells was not observed deeper in the dermis.

In the six-hour lesions the epidermal cells of some specimens show slight proliferating activity. The infiltration of the middle dermis has increased, and an intensive extravasation of the mononuclear cells is in progress. In the uppermost dermis the number of infiltrating cells has also increased; however, ascending mononuclear trails between the superficial capillaries and the epidermis, as well as the formation of vacuoles, are readily discernible. Singly or in groups, escorted by vacuoles, some mononuclears have entered the epidermis (Fig. 4).

In the nine- and twelve-hour lesions the trails of mononuclear cells can no longer be identified, since the diffuse cellular infiltration now extends into

the uppermost dermis. The epidermis has become slightly acanthotic (that is, from the original thickness of two to four cells to about three to six epidermal cells) and contains a number of regions invaded by groups of mononuclear

Fig. 1. Fig. 2.

Fig. 3. Fig. 4.

Fig. 1.—Three-hour lesion; 0.01 per cent DNCB. A trail of mononuclear cells. Note formation of vacuoles adjacent to the cells which are in proximity to the epidermis. (Magnification, ×400.)

Fig. 2.—Three-hour lesion; 0.1 per cent DNCB. Extravasation of mononuclear cells. Note on the left a linear arrangement of infiltrating cells ascending toward epidermis, some escorted by a vacuole. (Magnification, ×400.)

Fig. 3.—Three-hour lesion; 0.01 per cent DNCB. A capillary in the upper dermis with mononuclears crowding the vessel and leaving the lumen. (Magnification, ×400.)

Fig. 4.—Six-hour lesion; 0.1 per cent DNCB. Infiltration of the upper dermis with an ascent of mononuclear cells from a superficial capillary into the epidermis. Note vacuoles escorting the infiltrating cells. (Magnification, ×400.)

cells, accompanied by vacuoles of varying sizes (Figs. 5 to 10). Epidermal cells at the points of the mononuclear incursion seem only passively involved, having become disunited from their close cohesion. In some instances an intercellular edema spreads centrifugally into the surrounding epidermis (Figs. 7 and 8).

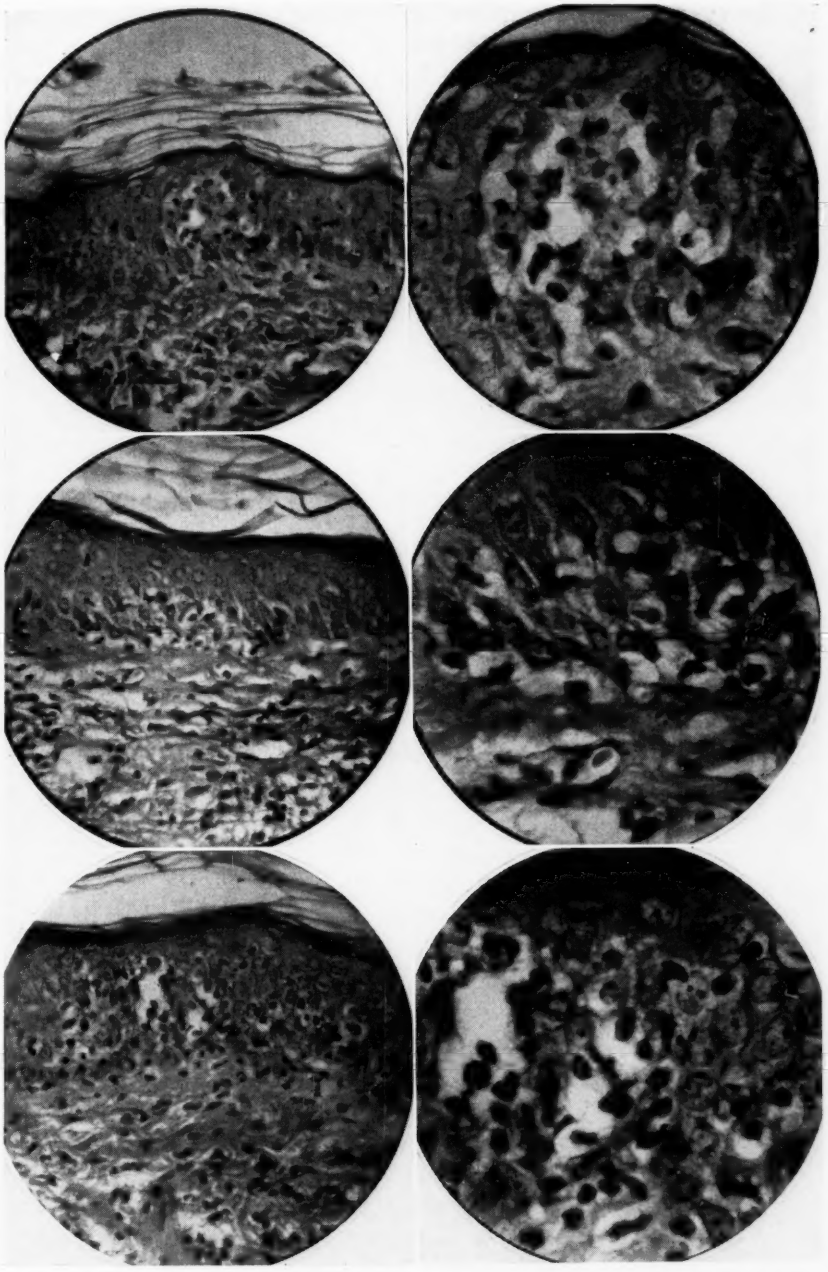


Fig. 5.

Fig. 6.

Fig. 7.

Fig. 8.

Fig. 9.

Fig. 10.

Figs. 5 and 6.—Nine-hour lesion; 0.01 per cent DNCB. A circumscribed invasion of the epidermis by mononuclear cells and formation of vacuoles. (Magnification: Fig. 5, ×400; Fig. 6, ×1,000.)

Figs 7 and 8.—Twelve-hour lesion; 0.1 per cent DNCB. A localized invasion of the epidermis by mononuclear cells. Formation of vacuoles at basal membrane and spread of intercellular edema in the epidermis. (Magnification: Fig. 7, ×400; Fig. 8, ×1,000.)

Figs. 9 and 10.—Twelve-hour lesion; 0.1 per cent DNCB. An epidermal region invaded by a number of mononuclear cells. Note varying stages of vacuole formation. (Magnification: Fig. 9, ×400; Fig. 10, ×1,000.)

In the twenty-four-hour lesions a formation of microvesicles was noted (Fig. 11). Examination of serial sections yielded the impression that the vesicles had developed at the points of a massive mononuclear cell invasion in a relatively small area of the epidermis (Figs. 12 to 14). Histologic changes in the periphery of the vesicles resemble the changes viewed in the nine- and twelve-hour lesions, that is, incursion of the epidermis by mononuclear, formation of vacuoles, and centrifugally spreading intercellular edema-spongiosis (Fig. 15). The tangential arrangement of the flattened epidermal cells (Fig. 14) seen in some sectors of the vesicles suggests that high central pressure is the mechanism of vesicle formation. On the other hand, spongelike multilocular sectors in the same vesicles (Fig. 11), formed by septa from flattened epidermal cells between individual vacuoles, indicate fusion of the vacuoles as the mechanism of vesicle formation.

Induration of the dermis reaches a maximum in the twenty-four-hour lesions. Interstitial edema and mononuclear cells diffusely distributed among dissociated collagen fibrils account for the two- to threefold thickening of the dermis (Fig. 16).

Histologic examination of biopsies made with a punch key or of those obtained from lesions folded and pinched between the fingers always revealed minute hemorrhages and a small number of polymorphonuclear cells. Apparently, this poor technique caused vascular injury and distortion of the infiltration. However, in the biopsies obtained by the scissor-snip method,[32] neither hemorrhages nor polymorphonuclears are seen.

The localized changes in the epidermis described above were not present in all twenty-four-hour lesions. They were observed in nearly one-half of the biopsies induced at this interval and were reproduced in the same animals weeks later. The change in the remaining biopsies was a rather general invasion of large regions of the epidermis by mononuclear cells accompanied by vacuoles, with or without formation of microvesicles (Fig. 17).

Epidermal changes reached a maximum development in forty-eight-hour lesions. All of the challenged epidermal surface is extensively invaded by mononuclear cells. Distinction between epidermis and dermis, easily distinguished in the stained sections, is difficult in black and white reproductions as a result of the density of the mononuclear infiltration and the total disruption of the epidermal structure by countless vacuoles. Marked vacuolization pervades the entire thickness of the epidermis (Fig. 18) and the upper portion of the hair follicles (Figs. 19, 20, and 21). Microvesicles were found in about one-half of the biopsies and were most prevalent in specimens with the greatest acanthosis. (No acanthosis was found in control biopsy specimens tested with plain olive oil.)

The changes observed in twenty-four-hour lesions were of two kinds—a circumscribed invasion and a general invasion of the epidermis by mononuclear cells. The changes in forty-eight-hour lesions largely resemble one another, being of one type only, an intensive general mononuclear invasion of the epidermis. It seems in order to assume that the varying epidermal changes observed

Fig. 11.

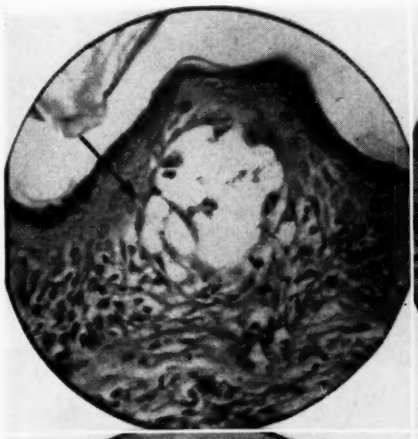


Fig. 14.

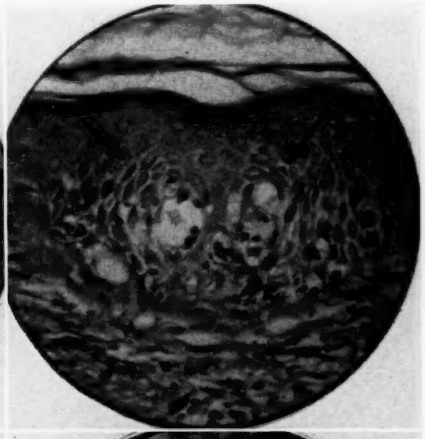


Fig. 12.

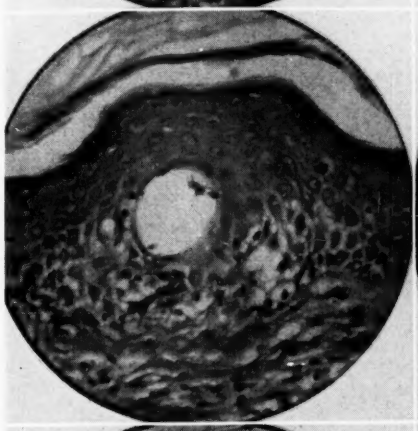


Fig. 15.

Fig. 13.

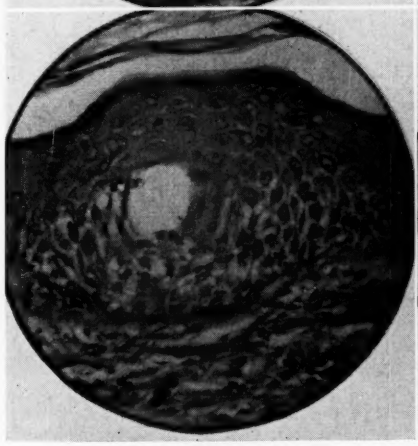


Fig. 16.

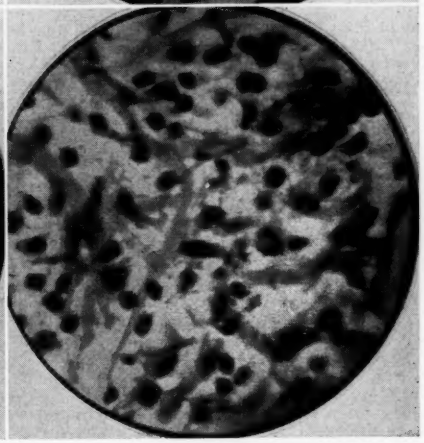


Fig. 11.—Twenty-four-hour lesion; 0.1 per cent DNCB. Central section of a microvesicle. Note spongelike, multilocular sector, formed by septa from flattened epidermal cells between individual vacuoles. (Magnification, ×400.)

Figs. 12, 13, and 14.—Twenty-four-hour lesion; 0.1 per cent DNCB. Peripheral sections through the microvesicle shown in Fig. 11. Note the tangential arrangement of epidermal cells surrounding the vesicular cavity. (Magnification, ×400.)

Fig. 15.—Twenty-four-hour lesion; 0.1 per cent DNCB. Detail of the microvesicle section shown in Fig. 14. Note mononuclear cells, varying stages of vacuole formation and the intercellular edema-spongiosis. (Magnification, ×1,000.)

Fig. 16.—Twenty-four-hour lesion; 0.1 per cent DNCB. Mononuclear infiltrating cells and edema of the upper dermis. (Magnification, ×1,000.)

Fig. 17.

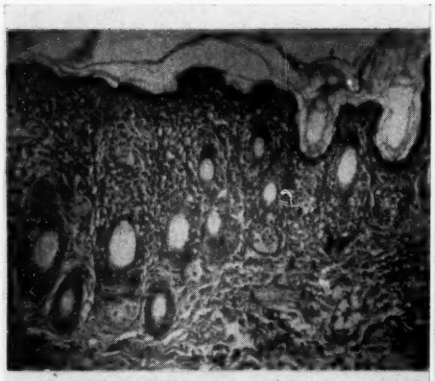


Fig. 20.

Fig. 18.

Fig. 21.

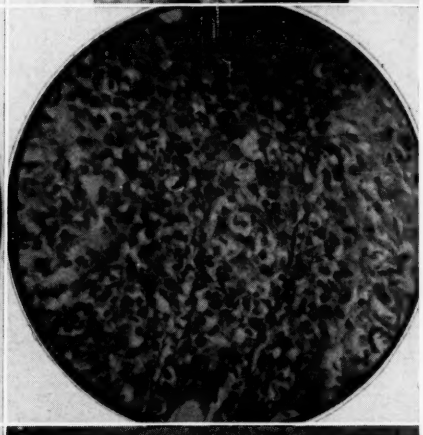


Fig. 19.

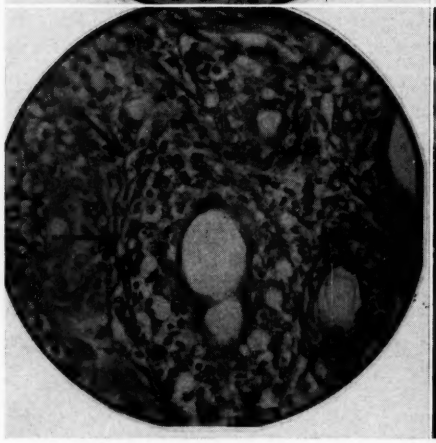


Fig. 22.

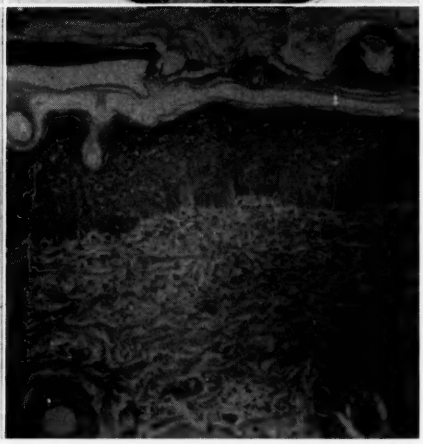


Fig. 17.—Twenty-four-hour lesion; 0.1 per cent DNCB. Generalized invasion of the epidermis and hair follicles by mononuclear cells and vacuoles. (Magnification, ×100.)

Figs. 18 and 19.—Forty-eight-hour lesion; 0.1 per cent DNCB. Massive mononuclear infiltration and marked vacuolization pervading the entire thickness of the epidermis (Fig. 18) and the upper portion of the hair follicles (Fig. 19, in cross section) totally disrupting the cohesion of epidermal cells. (Magnification, ×400.)

Fig. 20.—Forty-eight-hour lesion; 0.01 per cent DNCB. Hair follicle in longitudinal section. Invasion of the epidermal lining by mononuclear cells and vacuoles. (Magnification, ×100.)

Fig. 21.—Forty-eight-hour lesion; 0.01 per cent DNCB. Detail of Fig. 20. Massive mononuclear infiltration and marked vacuolization of the upper portion of the hair follicle lining. (Magnification, ×400.)

Fig. 22.—Five-day lesion; 0.1 per cent DNCB. Regenerated epidermis with hyperplasia of stratum granulosum and stratum malpighii, covered by a thin scale of cast-off epidermis containing mononuclear cell detritus. (Magnification, ×100.)

in twenty-four-hour lesions reach a uniformity and a maximum in forty-eight-hour lesions. In causal relationship, it is noteworthy that in the twenty-four-hour lesions the cellular infiltration has reached a maximum and is uniformly diffuse throughout the dermis. In the forty-eight-hour lesions the infiltrating cells crowd the epidermis and the upper portion of the dermis, whereas the deeper layers of the dermis now show only a moderate infiltration. The observed shift in density of cellular infiltration is a result of a transmigration of the infiltrating cells toward and into the epidermis. Therefore, the two types of changes seen in the epidermis of twenty-four-hour lesions seem to be related to the velocity of locomotion, the distance which the infiltrating cells must travel, or both. In the lesions of following days the number of infiltrating cells in the dermis remains constant, whereas the accompanying vasodilatation and interstitial edema gradually disappear.

Fig. 20.

In the three-day lesions the vacuoles near the epidermal basement membrane have fused, separating the epidermis and the dermis. The staining property of the epidermal layer is altered, for it stains poorly with hematoxylin and the affinity for eosin is not increased. In the four-day lesions the dead epidermis and the fragments of mononuclear cells are shed. A new epidermal layer (1 to 3 cells thick) covers the dermis. These new cells show proliferation and normal staining property. The probable location of epidermal cells from which the regeneration of the epidermis has begun was not determined.

Fig. 21.

The five-day lesions show marked thickening of the new epidermis due to hyperplasia of the stratum granulosum and stratum malpighii. The epidermal surface is covered by a thin scale of cast-off epidermis with fragments of mononuclear cells (Fig. 22).

Fig. 22.

Histologic Changes at the Site of Primary Toxic Dermatitis.—Three hours after a toxic concentration (2 per cent, 3 per cent, and 5 per cent) of DNCB in olive oil has been applied to the shorn skin of normal animals, neither the epidermis nor the dermis shows any histologic change. Epidermal changes occur in a few six-hour lesions but are manifest in all twelve-hour lesions. The histologic findings vary with the concentration of the chemical and also with each animal. To simplify description, the predominant changes are enumerated as observed in lesions induced by the different concentrations of DNCB.

In lesions induced by 5 per cent DNCB the predominant toxic damage is an epidermal necrobiosis, in varying stages of development from incomplete (Fig. 24) to complete (Fig. 23) (loss of cellular integrity and of the distinction between nucleoplasm and cytoplasm). Irrespective of the stage of epidermal necrosis, a separation of the epidermis from the dermis was seen in all specimens. In lesions induced by lesser concentrations of DNCB the epidermal damage was less marked. Basal-cell layer proliferation with simultaneous epidermal separation is prevalent in lesions induced by 3 per cent DNCB (Fig. 25), whereas proliferation without epidermal separation occurs in lesions induced by 2 per cent DNCB. The dermis in the twelve-hour lesion is moderately indurated by interstitial edema and polymorphonuclears and mononuclears in about the same proportion as found in blood.[32]

Fig. 23.

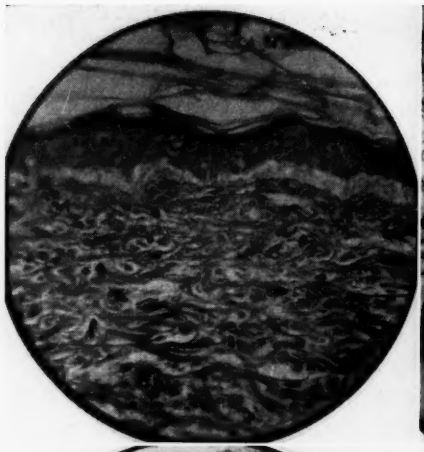


Fig. 26.

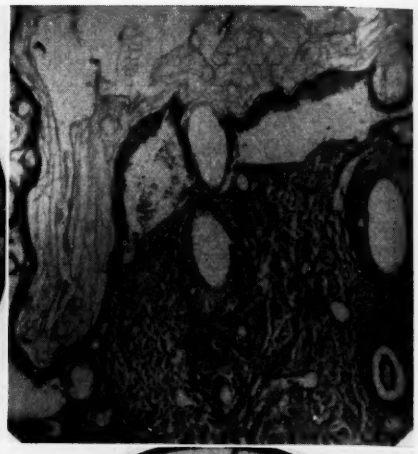


Fig. 24.

Fig. 27.

Fig. 25.

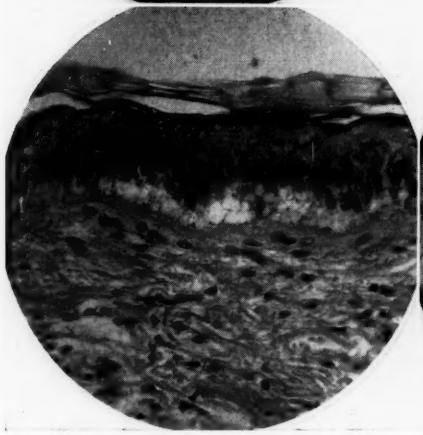


Fig. 28.

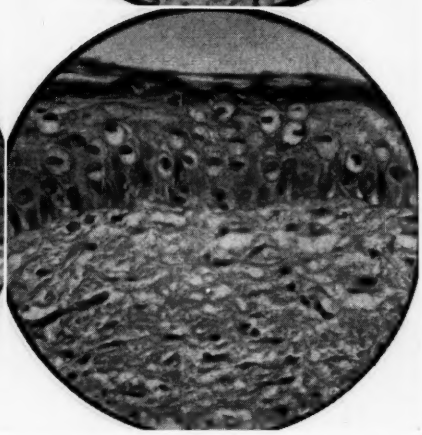


Fig. 23.—Twelve-hour lesion; 5 per cent DNCB. Complete epidermal necrosis (loss of integrity of individual cells and the distinction between nucleo- and cytoplasm) and partial separation from the dermis. (Magnification, ×400.)

Fig. 24.—Twelve-hour lesion; 5 per cent DNCB. Partial epidermal necrobiosis and separation from the dermis. (Magnification, ×400.)

Fig. 25.—Twelve-hour lesion; 3 per cent DNCB. Proliferation of basal-cell layer and partial epidermal separation from the dermis. (Magnification, ×400.)

Fig. 26.—Twenty-four-hour lesion; 5 per cent DNCB. Total epidermal detachment with complete epidermal necrosis. (Magnification, ×100.)

Fig. 27.—Twenty-four-hour lesion; 3 per cent DNCB. Detachment of epidermis with partial epidermal necrobiosis. (Magnification, ×400.)

Fig. 28.—Twenty-four-hour lesion; 2 per cent DNCB. Generalized perinuclear vacuolization of epidermal cells. (Magnification, ×400.)

Fig. 29.

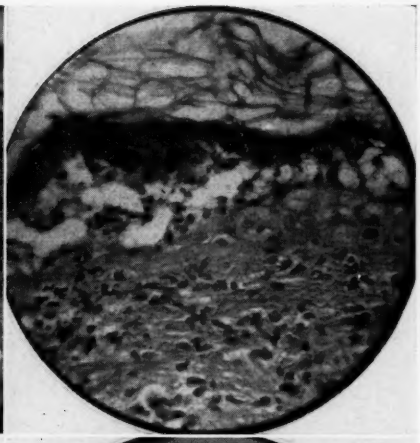


Fig. 32.

Fig. 30.

Fig. 33.

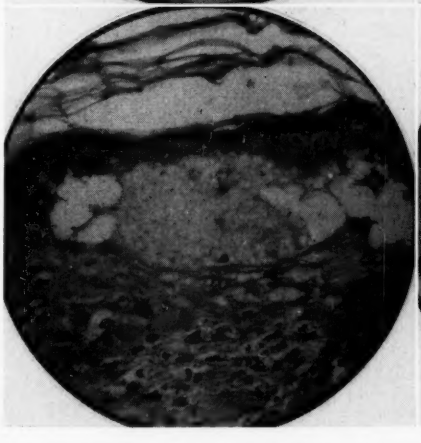


Fig. 31.

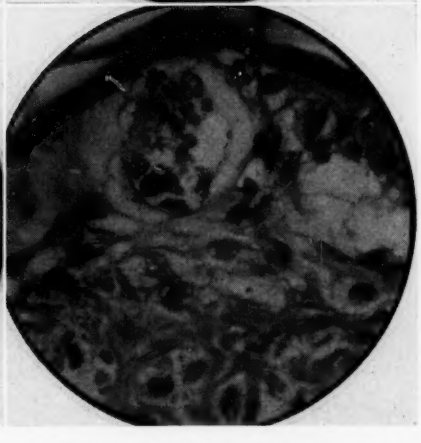


Fig. 34.

Fig. 29.—Twenty-four-hour lesion; 5 per cent DNCB. An intraepidermal cleavage at a junction of complete and partial necrobiosis of the epidermis. Note accumulation of inflammatory cells (polymorphonuclears and mononuclears) in the dermis and in the intraepidermal cleavage. (Magnification, ×100.)

Figs. 30, 31, and 32.—Forty-eight-hour lesions; 5 per cent DNCB. Details of intraepidermal cleavages. Note necrotic surface and surviving basal portion of the epidermis shaping the cavity containing disintegrated epidermal cells (Fig. 30), serous fluid (Fig. 31), and inflammatory cells, predominantly polymorphonuclears (Fig. 32). (Magnification, ×400.)

Figs. 33 and 34.—Forty-eight-hour lesion; 3 per cent DNCB. A circumscribed microvesicle at a junction of complete and partial necrobiosis of the epidermis. Note in the detail (Fig. 34) the content of the vesicle: cellular detritus and polymorphonuclear inflammatory cells. (Magnification: Fig. 33, ×400; Fig. 34, ×1,000.)

In twenty-four- and forty-eight-hour lesions the epidermal changes are as manifold as in twelve-hour lesions. However, more or less definite patterns of epidermal destruction were identified: (1) total detachment of epidermis in specimens with complete epidermal necrosis (Fig. 26), (2) partial detachment with incomplete epidermal necrobiosis (Fig. 27), (3) generalized perinuclear vacuolization of the epidermis (Fig. 28), and (4) formation of intraepidermal cleavages containing inflammatory elements (Fig. 29). The first three patterns seem to evolve from the changes observed in twelve-hour lesions. However, the formation of intraepidermal cleavages seems to be a variation of these changes which arises later (twenty-four to forty-eight hours). Without exception, a necrotic surface portion and a surviving basal portion of the epidermis shape the intraepidermal cleavages (Figs. 30 to 32), within which are found disintegrated epidermal cells (Fig. 30), serous fluid (Fig. 31), and inflammatory cells, predominantly polymorphonuclears (Fig. 32). The dermal infiltration by inflammatory cells reaches a maximum in the same lesions (twenty-four to forty-eight hours). A slow disintegration of the epidermis and migration of inflammatory elements from the dermis seem to be the prerequisites for development of the intraepidermal inflammatory reaction. In some specimens the intraepidermal inflammatory process gives rise to microvesicles (Figs. 33 and 34). A close examination of the vesicle reveals the same basic structure as described above, namely, location at a junction of complete and partial epidermal necrobiosis, containing cellular detritus and inflammatory cells, predominantly polymorphonuclears.

In a few forty-eight-hour lesions, induced by 5 per cent DNCB, the toxic damage extends into the uppermost portion of the dermis. The damage manifests itself with degenerative fragmentation of collagen fibrils next to the necrotic epidermis.

A reparative process first becomes apparent from the histologic changes of three-day lesions. The reproduction of cells is noticeable in the epidermal lining of the upper part of the hair follicles (Fig. 35). The epidermal cells reach the follicular orifice and spread centrifugally under the slough (Fig. 36). In four-day lesions a regenerated epidermal layer, two to three cells thick, covers the entire dermal surface and is overlaid with the dead epidermis (Figs. 37 and 38). In the five-day lesions the new epidermal layer becomes five to eight cells thick (Fig. 40). The basal cells proliferate and the surface cells show degeneration (Fig. 41).

Regeneration of the epidermis is faster than that of the collagen fibrils, first noted in a few forty-eight-hour lesions. A small number of mononuclear cells appear on the fourth day among the fragments of the fibrils (Fig. 39). In the seven- and nine-day lesions the collagen fibrils are largely restored. Residual fragmentation and the presence of pale fibrocytes among the restored fibrils point out the antecedent condition (Fig. 42).

Repair of the collagen is overlapped in time by another histologic reaction, noted first in six- and seven-day lesions. The reaction is characterized by a

Fig. 35.

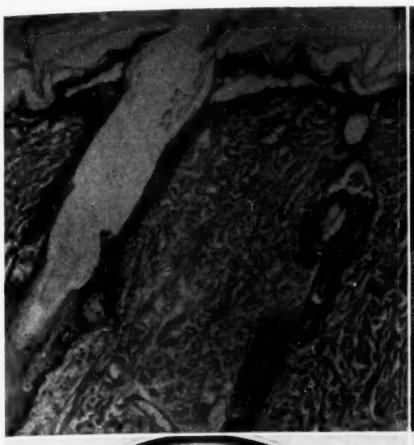


Fig. 37.

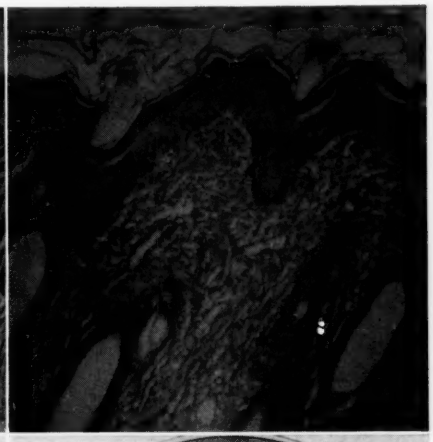


Fig. 36.

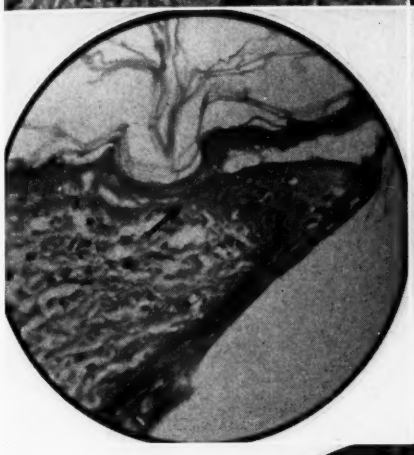
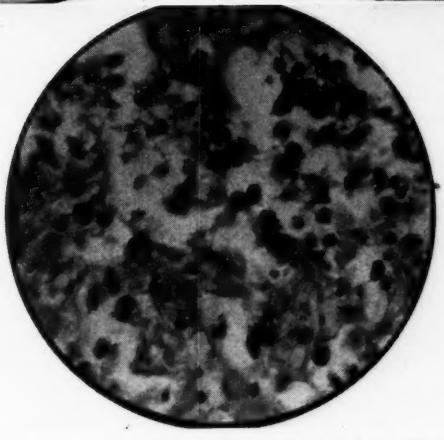


Fig. 38.

Fig. 39.

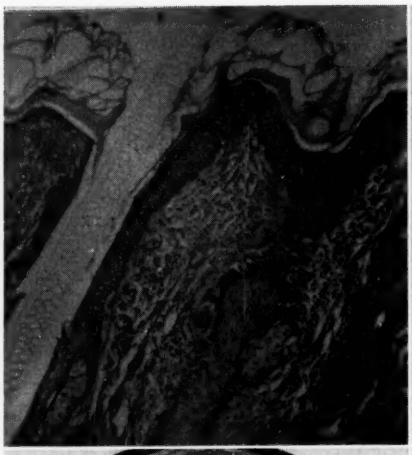


Figs. 35 and 36.—Three-day lesion; 5 per cent DNCB. The regeneration of the epidermis begins in the upper portion of the hair follicle. Note an island of epidermal cells around the follicular orifice (Fig. 36) spreading between the dermis and the dead epidermis. (Magnification: Fig. 35, ×100; Fig. 36, ×400.)

Fig. 37.—Four-day lesion; 5 per cent DNCB. Regenerated epidermis, four cells thick, covers the dermal surface and is overlaid by the toxic slough. (Magnification, ×100.)

Figs. 38 and 39.—Four-day lesion; 5 per cent DNCB. Detail of Fig. 37. Note the fragmentation of the collagen fibrils of the uppermost dermis with an accumulation of minute mononuclear cells. (Magnification: Fig. 38, ×400; Fig. 39, ×1000.)

Fig. 40.



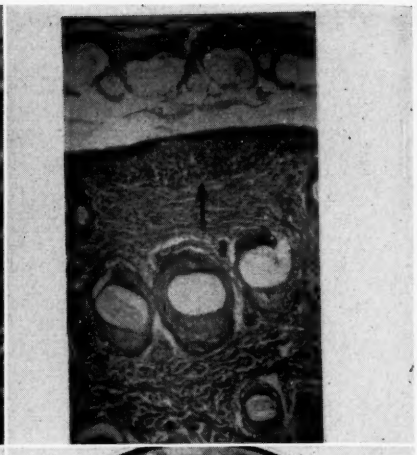


Fig. 41.

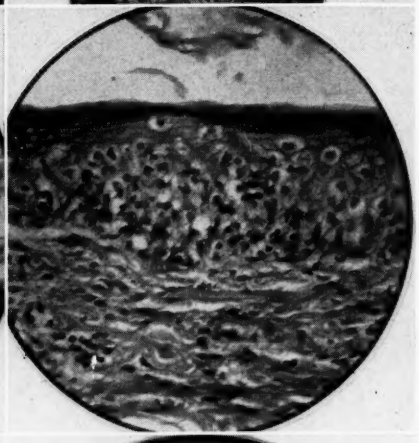
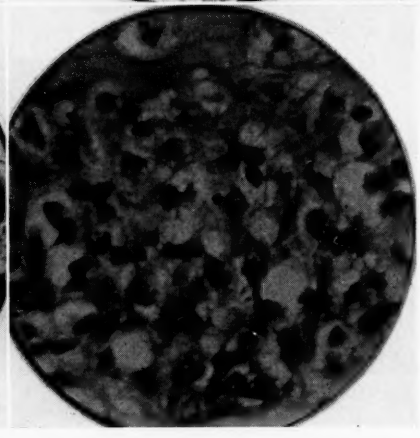


Fig. 42.



Figs. 40 and 41.—Five-day lesion; 3 per cent DNCB. Regenerated epidermis, six to eight cells thick. The epidermis is overlaid with a slough. The basal cells proliferate, whereas the upper epidermal strata show dyskeratotic vacuolization. (Magnification: Fig. 40, ×100; Fig. 41, ×400.)

Fig. 42.—Six-day lesion; 5 per cent DNCB. The restoration of damaged collagen fibrils. Note the pale-staining fibrocytes between the collagen fibrils. (Magnification, ×1,000.)

Fig. 43.—Seven-day lesion; 5 per cent DNCB. Regenerated and acanthotic epidermis beneath a slough of toxic reaction. Note invasion of the regenerated epidermis by infiltrating cells. (Magnification, ×100.)

Figs. 44 and 45.—Seven-day lesion; 5 per cent DNCB. Details of Fig. 43. Some epidermal surface cells show a dyskeratotic degeneration. The lower portion of the epidermis is massively invaded by mononuclear leukocytes. Note in Fig. 45 the vacuoles adjacent to mononuclear cells. (Magnification: Fig. 44, ×400; Fig. 45, ×1000.)

flare-up of capillary dilatation, increase of interstitial edema, a fresh extravasation of mononuclear cells into the perivascular tissue, and an invasion of the acanthotic epidermis by mononuclear cells (Fig. 43). The histologic change in the epidermis at the point of invasion of mononuclear cells (Figs. 44 and 45) is identical with that seen in early lesions of allergic contact dermatitis. A formation of microvesicles occurred in most of the nine-day lesions. This histologic change in the six-, seven-, and nine-day lesions coincides with the development of general allergic sensitivity determined by epicutaneous test with 0.75 per cent DNCB in olive oil in a new area of the skin. It seems that the antigen left at the toxic side induced an allergic flare-up at the site of the primary lesion, thus giving rise to histologic changes characteristic of allergic contact dermatitis of extremely sensitive animals. However, the biopsies obtained from the allergic test lesions, induced by 0.75 per cent DNCB in the same animals, revealed only a dense dermal infiltration by mononuclear cells. The epidermal changes seen in the flaring toxic lesions were not reproducible. Various factors present at the site of the original toxic lesion could be responsible for the epidermal reaction. Among these factors are acanthosis of the regenerated epidermis[15, 17] and vascularization of dermis, both subsequent to toxic injury, which constitute the difference in anatomic structure between the original toxic lesion and the freshly shorn area of skin used for the epicutaneous test.

DISCUSSION

The histologic study of allergic and toxic dermatitis was undertaken not only with the object of determining the changes characteristic of both types but also with the thought of finding a clue to the mechanism of the allergic response.

The histologic changes in the toxic and allergic lesions are strikingly different, although the duration of the two reactions is about the same (five to six days) and in both the peak of injury is reached at about forty-eight hours.

The three- to twelve-hour lesions of *toxic dermatitis* are characterized by epidermal injury from withering of the epidermal cells to total necrosis, always accompanied by scant extravasation of polymorphonuclear and mononuclear cells. The three- to twelve-hour lesions of *allergic dermatitis* are characterized by a massive extravasation of mononuclear cells migrating in trails directly into the epidermis. In the toxic reaction the concentrated (5 per cent) chemical caused relatively sparse and scattered cellular infiltration; in the allergic reaction a dilute concentration (0.01 per cent and 0.1 per cent) of the chemical induced prompt extravasation and direct ascent of the mononuclears toward the challenged skin surface. *The most plausible explanation for the behavior of the mononuclear cells seems to be that the sensitization has induced in them a capacity for positive chemotaxis; that is, a faculty to detect a chemical (or its protein derivative) in minute concentrations and to migrate straight into the area of its highest concentration.*[36]

Nearly all *toxic lesions* of twenty-four to forty-eight hours showed an increase of polymorphonuclear and mononuclear cell infiltration in the dermis following the earlier death of epidermal cells. (In some, in which the epidermis

had partially survived, a formation of intraepidermal cleavages containing infiltrating cells was observed. Partial epidermal necrosis with simultaneous increase of cellular infiltration of the dermis seems to be a requisite condition for this development. It appears that the slow toxic disintegration of the epidermis has promoted the transmigration of infiltrating cells into the epidermis.[37]) The *allergic lesions* of twenty-four to forty-eight hours, on the other hand, showed a massive invasion of the epidermis by mononuclear cells and formation of vacuoles. Examination of serial sections revealed that each vacuole adjoins at least one mononuclear cell. In some lesions microvesicles develop, presumably at points where vacuoles had fused. The vacuolization and vesiculation cause a disruption of the epidermis which apparently lessens

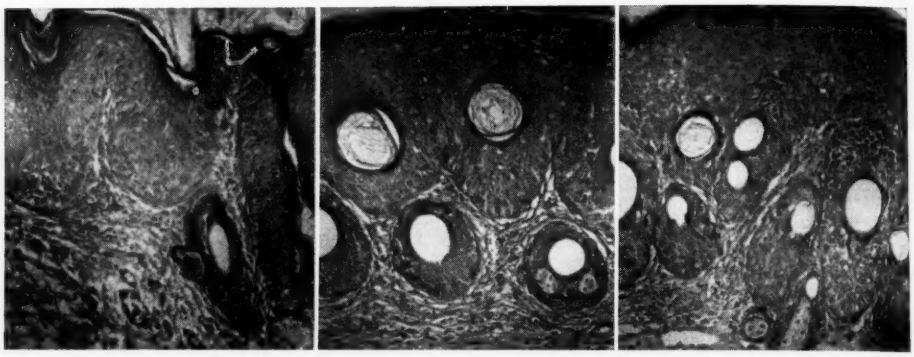


Fig. 46.—Forty-eight-hour lesions; 0.1 per cent DNCB. Three lesions of allergic contact dermatitis of highly sensitive guinea pigs injected with germanin. (100 mg. germanin subcutaneously daily for five days; epicutaneous challenge with DNCB on the third day.) Note the absence of mononuclear-cell invasion into the extremely thickened epidermis. Control sites tested with plain olive oil showed normal histology. (Magnification, ×100; reduced 1/10.)

its viability and leads to its death and exfoliation. *Mononuclear cell invasion, vacuolization, vesicle formation, and exfoliation of the epidermis are steps in a process characteristic of the allergic response not seen in the toxic lesions.* An excess of histamine accumulates at the site of allergic DNCB contact dermatitis only in the presence of mononuclear cells.[32, 38] This accumulation results from activation of histamine metabolism by promotion of histidine decarboxylation. Administration of germanin (decarboxylase inhibitor) was found to inhibit the histamine formation.[32] Furthermore, it has been shown that the infiltrating cells in allergic DNCB reaction have an increased activity of alkaline phosphatase, which is inhibited by administration of heparin.[39] In the toxic lesions there is neither histamine accumulation[32] nor increased phosphatase activity.[39] *Thus the allergic reactions show not only a specific cellular response but also a characteristic intensity in local metabolic activity, enzymatic in character.*

These results suggest that the reaction of the allergic contact type is dependent on mononuclear leukocytes which acquire specific capacities through sensitization. However, histologic findings at sites of allergic dermatitis of guinea pigs injected with germanin are indicative of a sensitization of epidermal cells as well. In all animals injected with germanin, the forty-eight-hour

allergic lesions reveal considerable dermal infiltration by mononuclear cells. However, the epidermis is extremely thickened and not invaded by the mononuclears (Fig. 46). It seems that germanin has prevented ascent of mononuclear cells in the epidermis. *The extreme epidermal thickening might be regarded as the allergic response of epidermal cells after the participation of mononuclears has been forestalled.*

SUMMARY

Chronologic development of histologic changes at the site of allergic and toxic dermatitis is described in detail.

Mononuclear cell invasion, vacuolization, vesicle formation, and exfoliation of the epidermis are steps in a process characteristic of the allergic response.

A comparison of the histologic and chemical changes in toxic and allergic dermatitis indicates that sensitization induced a capacity in the mononuclear cells for positive chemotaxis, for induction of characteristic inflammatory changes, and for participation in metabolic processes (presumably by increased enzymatic activity).

A sensitization of epidermal cells has not been ruled out. An extreme acanthosis seen in allergic lesions of animals treated with germanin might be regarded as the allergic response of epidermal cells after participation of mononuclear cells has been forestalled.

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A QUANTITATIVE STUDY ON THE IN VITRO RELEASE OF HISTAMINE FROM LEUKOCYTES OF ATOPIC PERSONS

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HISTAMINE release from leukocytes can be induced in vitro when an antigen, such as a pollen extract, is incubated with the blood of a person with the appropriate atopic sensitivity. This phenomenon was first reported by Katz and Cohen[1] seventeen years ago and recently has been studied carefully under different conditions by Noah and Brand,[2, 3] who utilized a sensitive microchemical assay for histamine developed by Lowry and colleagues.[4]

Noah and Brand[3] found that release of histamine could be obtained in atopic blood exposed to a variety of allergens, including pollens, molds, danders, foods, and house dust. The only requirement seemed to be the presence of the appropriate skin-sensitizing antibody in serum of the blood tested.

Studies of the mechanisms by which the human allergic reaction is mediated have been hampered in the past by the lack of any in vitro system which could be used for measuring the effects of the atopic reagin. In particular, it has not previously been possible to gauge accurately the effects of combining varying amounts of antigen with the skin-sensitizing antibody. For this reason, we have studied particularly the relationship between the amount of antigen added to allergic blood and the amount of histamine released into plasma as a result.

METHODS

Blood Donors.—These were persons with clinical evidence of atopic disease whose sensitivity was confirmed by intracutaneous tests. All of them were in good health, many being medical students or laboratory personnel. With a few exceptions, the blood was obtained at a time when the person was asymptomatic. None of these persons had received previously any antigen injections for the purpose of desensitization.

A majority of persons were strongly sensitive either to ragweed or to timothy pollen. The remainder were sensitive to tree or English plantain pollens or were laboratory personnel who had developed allergic symptoms on exposure to experimental animals.

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Blood Collection.—In all instances, venepuncture was made with a No. 15 needle, and the blood was allowed to drain without suction through a short length of Tygon tubing (inner diameter 3/8 inch) into 40 ml. siliconed* centrifuge tubes, each containing 0.5 mg. (50 units) of nonphenolized sodium heparin. Twenty-five to 40 ml. aliquots were collected in each tube, which was then gently and briefly shaken. From 200 to 300 ml. of blood was thus collected at one time.

Antigens.—All preparations were standardized for protein nitrogen content and diluted shortly before use. The ragweed antigens were diluted with 0.9 per cent sodium chloride; the most concentrated solutions in earlier experiments contained 0.5 per cent phenol. All other antigens were prepared and diluted with phosphate-buffered saline (pH 7.8). The animal dander extracts were obtained as bulk concentrates in 50 per cent glycerin-buffered saline,† assayed for protein nitrogen content, and appropriately diluted with buffered saline.

Additions of Antigens and Incubation.—The antigen solutions were prepared to contain varying concentrations from 100 to 0.00025 mcg. protein nitrogen (P.N.) per milliliter. One milliliter of each concentration to be tested was added to one 25 or 40 ml. aliquot of freshly collected blood. Usually four to eight antigen concentrations were selected and studied at one time.

After the antigens were added, all blood specimens, including an extra aliquot containing 1 ml. of diluent alone, were placed in an incubator at 37° C., gently stirred every five minutes, and removed in most instances after thirty minutes.

Assay of Histamine.—After incubation, all samples were centrifuged at 2,500 r.p.m. for fifteen minutes. The supernatant plasma was removed, and one fifth volume of 30 per cent trichloroacetic acid was added to this plasma to precipitate the plasma proteins. After each blood collection, aliquots of whole blood were set aside for determination of total blood histamine. An equal volume of 10 per cent trichloroacetic acid was added to the whole blood specimens to precipitate proteins and release all intracellular histamine. After the trichloroacetic acid-treated specimens were centrifuged, measured aliquots of the clear supernatant solutions were removed, buffered with one tenth volume of 4 M sodium acetate, and analyzed chemically for histamine by the method of Lowry and associates.[4] The histamine released into plasma was then calculated in terms of micrograms of histamine base per liter of plasma.

Determination of the Rate of Histamine Release.—In order to obtain more information concerning the nature of the histamine release phenomenon, we wished to determine the rapidity of onset of histamine release and the nature of the release versus time relationship. The extra time necessary for centrifugation obviated any direct observations on such release after short intervals of time. Fortunately, it was noted that the addition of 100 mg. sodium ethylene-

*Desicote, a chloroform solution of silicone, obtained from Beckman Instruments Inc., Fullerton, California, was used for this purpose.

†Obtained from Hollister-Stier Laboratories, Spokane, Washington.

diamine tetraacetic acid (E.D.T.A.)* to the antigen-containing blood would completely block release of histamine. Accordingly, blood was again obtained in several aliquots from appropriately sensitive persons, and identical amounts of pollen extract (in concentrations producing optimal histamine release) were added to each aliquot. The E.D.T.A. was then added to successive aliquots in duplicate at 0, 1, 2, 5, 10, 15, 30, and (in one experiment) 60 minutes following addition of antigen. All aliquots were incubated for the same period of time and centrifuged, the supernatant plasma again being assayed for histamine content.

RESULTS

The levels of total blood histamine, control plasma histamine, and plasma histamine following incubation with varying amounts of antigen are presented in Table I. In a few instances, release of histamine was effected by as little as 0.01 mcg. protein nitrogen (P.N.) of antigen per liter of blood (0.001 protein nitrogen units per milliliter of blood). Conversely, release of histamine did not occur at all in a few instances, although blood was obtained only from persons with clear-cut clinical sensitivity and positive skin tests (except for Subject 33).

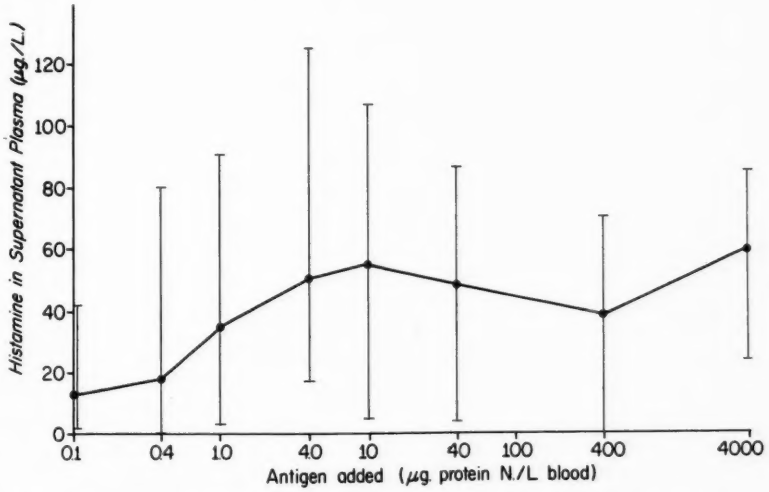

Fig. 1.—Histamine in supernatant plasma following incubation of whole blood with varying amounts of specific antigen. These values are the means of those obtained in twenty-nine separate experiments.

Maximal histamine release occurred at various concentrations of antigen, in most cases from 4 to 20 mcg. P.N. per liter of blood. Fig. 1 shows the relationship of histamine release to antigen concentration. The values given represent the means and extreme values for those experiments in which histamine release occurred and adequate data were obtained. Accordingly, data on Subjects 4, 16, 19, 20, 21, 29, 31, 32, 33, 36, 39, and 41 (Table I) were not included. The drop in histamine release which occurred when the concentration

*Kindly supplied as disodium Versenate by Dr. George Berryman of the Abbott Laboratories, North Chicago, Illinois.

TABLE I. HISTAMINE RECOVERED FROM PLASMA AFTER INCUBATION OF BLOOD OF VARIOUS SENSITIVITIES WITH APPROPRIATE ANTIGENS (VALUES EXPRESSED AS MCG. HISTAMINE BASE PER LITER OF PLASMA)

SUBJECT	ANTIGEN	SKIN TEST (0.03 MCG. P.N.)	WHOLE BLOOD HISTAMINE* (MCG./L.)	ANTIGEN CONCENTRATION (MCG. P.N./LITER WHOLE BLOOD)																
				0	0.01	0.02	0.04 0.05	0.10	0.20	0.40 0.50	1.0	2.0	4.0 5.0	10	20	40 50	250	400	4000
1	Ragweed	3+	49	0	0		0	5		12	20	30	38	36	37				
2	Ragweed	4+	80	3	12	15		20	22		23	27	32	35					
3	Ragweed	4+	217	24	29	37		42	48		49	58	52	56					
4	Ragweed	4+	78		16	16	13	15	12		16	21							
5	Ragweed	4+	121	11	12		7			6	26	36	51	49	50	46†			
6	Ragweed	3+	63	3	3		3			3	3		17	28	37	38†			
7	Ragweed	2+		4			5	9		11	16	20	61		61	69†			
8	Ragweed	4+	67	0			0	11		13	15	27	39	37	47	43†			
9	Ragweed	1+	77	3			5	3		10	15	25	41	56	70	12†			
10	Ragweed	3+	65	4				2		5	12	28		52		61†	43†		
11	Ragweed	4+	101	4								13	40			38†			
12	Ragweed	3+	61	5						5		12	25		43	44†	33†		
13	Ragweed	5+	93	6								15		74	83	87†	59†		
14	Ragweed	5+	99	4									42	48	48	36†	14†		
15	Ragweed	3+	109	0								6		42					
16	Ragweed	4+	120	0							0	10	27	45			39†		
17	Ragweed	5+	173	6								36	49	52	40	25†	20†		
18	Ragweed	5+	90	0			0			11			65			45		20	82
19	Timothy	2+	66	3			2	4		3	4	1	3	9		23†			
20	Timothy	4+	67	3						2	3		4		11	10†			
21	Timothy	3+	88	0									0‡			11‡		18‡	77‡
22	Timothy	4+	77	4			5			20			56			31		27	
23	Timothy	3+	82	11						54	71		78	82		71			
24	Timothy	4+	63	11			14			14	50		75	84		55		59	36
25	Timothy	5+		0						0	7		27	40		33		33	47
26	Timothy	4+	91	0			0			34	51		78	71		62		53	71
27	Timothy	2+	107	18			23			20	55		73	82		75		64	85
28	Timothy	5+	88	0			2			18	34		20	5		4		7	25
29	Timothy	4+	104	5									62						
30	Timothy	4+	62	5			7			20	20		51	51		33		20	40
31	Timothy	4+	113	24									134						
32	Timothy	5+	69	4									73	61		62			
33	Timothy	Non-allergic	75	13									13			14		13	26
34	Plantain	5+	160	4			20			80	91		125	107		76		53	76
35	Plantain	5+	44	0			0			7	27		56	51		18		0	44
36	Plantain	3+	99	9									15	15		15			
37	Alder	4+	116	14			14			35	73		87	100		73		71	71
38	Guinea pig	4+	104	5						10			17	29		62		47	82
39	Rat	3+	70	5						7						7		9	20
40	Rat	4+	69	15						11			45			73		51	
41	Rabbit	2+	79	9						9			15			10		11	

*Corrected for assumed 11 per cent nonhistamine substances in red cells.
†0.25 per cent phenol in blood.
‡0.25 per cent phenol in blood.

of antigen exceeded 10 mcg. P.N. per liter is noteworthy. The secondary rise at 4,000 mcg. P.N. per liter may be nonspecific, since histamine release from blood of nonallergic persons occurs at this concentration (see Subject 33).

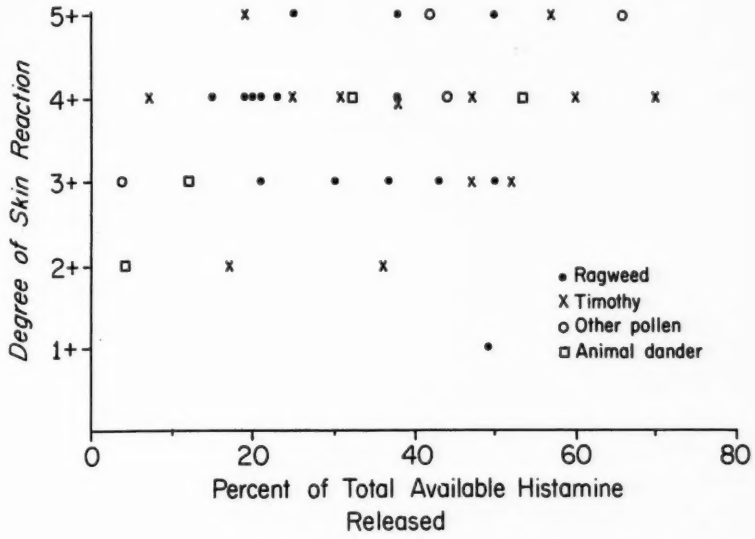

Fig. 2.—Maximum amount of histamine releasable by specific antigen, related to degree of skin reactivity (0.03 mcg. protein nitrogen given intracutaneously).

In Fig. 2 it can be seen that little correlation exists between the degree of skin reactivity and the percentage of total available histamine released. The figures in the abscissa were obtained from the following equation:

$$\text{per cent total available histamine released} = \frac{(\text{maximum plasma histamine})-(\text{control plasma histamine})}{\left(\frac{\text{total blood histamine}}{0.55}\right)-(\text{control plasma histamine})}$$

where 0.55 represents the fraction of plasma in whole blood.

Some relationship, however, could be shown between skin reactivity and the concentration of pollen extract which produced maximum histamine release. This is expressed in Fig. 3. It should be noted that the concentration of animal dander antigen producing maximum histamine release was higher in the two adequate studies (Subjects 38 and 40, Table I); these data were not included in Fig. 3. The dotted line was obtained by fitting the available data to an assumed exponential curve and corresponds to the formula $Y = 5.1 - 1.3 \log x$.

The rate of histamine release into the plasma of pollen-sensitive persons is indicated in Fig. 4. From the available data, it appears that this release occurs in a linear fashion, at least for the first fifteen minutes, and approaches an end point after thirty minutes of incubation. No attempt was made to establish an absolute end point.

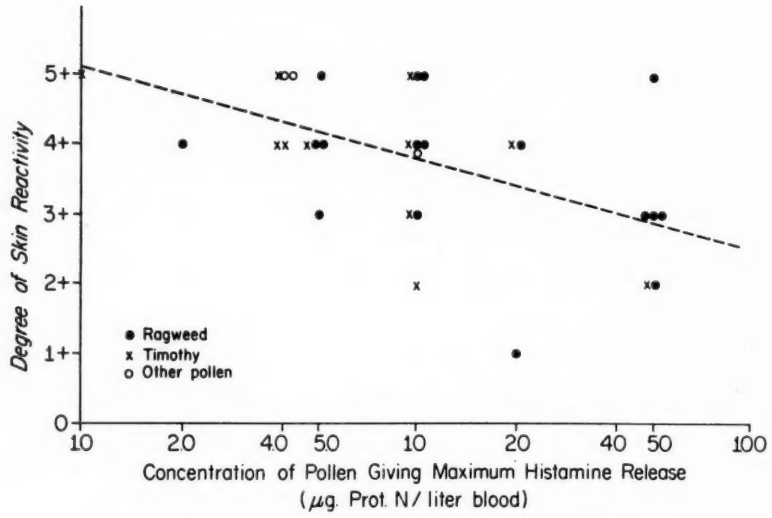

Fig. 3.—See text for further explanation.

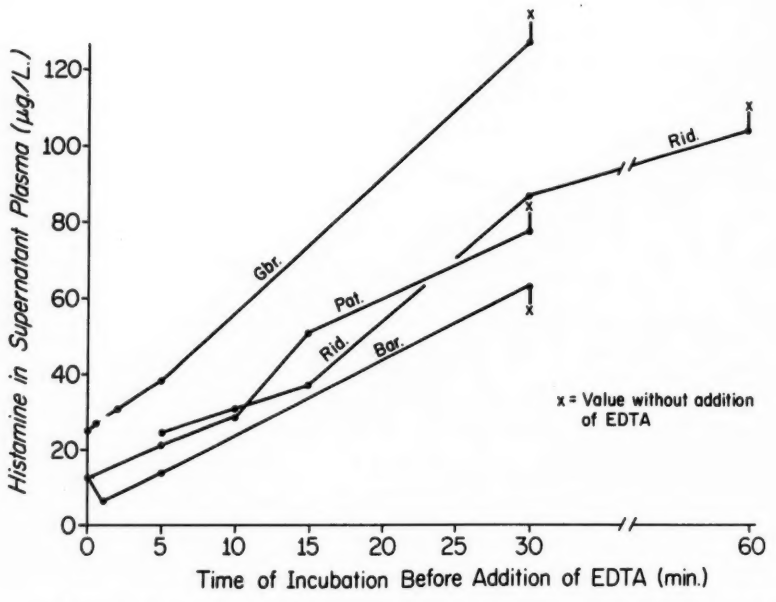

Fig. 4.—Relationship of histamine release to length of incubation of whole blood with specific antigen.

DISCUSSION

The release of histamine from its intracellular sites can be readily induced in vitro when antigen is added to whole blood from atopic persons. This phenomenon is antigen specific and cannot be produced in nonatopic blood[3] unless a very high concentration of antigen is used (Table I). This reaction is highly sensitive; at times as little as 10^{-2} meg. P.N. per liter of whole blood was sufficient to produce histamine release. In most instances release could be readily produced by antigen at a concentration of 1.0 meg. P.N. per liter of whole blood.

The molecular weight of pollen antigens is not known exactly, but estimates of from 14,000 (grass) to 18,000 (ragweed) have been reported.[5, 6] The fraction of total phosphotungstic acid-precipitable nitrogen in pollen antigens is also difficult to estimate; a reasonable figure seems to be 4.5 per cent, as reported by Frankel and associates.[7] By use of these figures, the molar concentration of crude pollen necessary for minimal histamine release would range between 1.6×10^{-3} and 1.2×10^{-5}, with maximal release (see also Fig. 1) occurring most frequently at a concentration of, roughly, 1.4×10^{-2} M. The effective molar concentration of the specific skin-reactive component of pollen would be much lower, but, in spite of extensive studies on pollen antigen fractionation,[7-10] even a rough estimate of what this concentration might be is impossible.

The great variation in response from person to person is noteworthy and could be related neither to skin reactivity nor to intensity of clinical symptoms. In spite of these variations, two patterns seemed clear. First of all, a clear-cut drop in the amount of histamine released was frequently observed as the concentration of antigen exceeded 10 meg. P.N. per liter of blood. This effect was quite striking in certain individual experiments (for example, subjects 14, 22, 26, 30, 34, and 37) but was sufficiently consistent to be demonstrable when all information was pooled (Fig. 1). Although the small amount of phenol present in extracts used in some of the early studies (Table I) might have produced some drop in histamine release, sufficient experiments were performed with extracts containing neither phenol nor glycerin to confirm the validity of this phenomenon.

This inhibitory effect of excess antigen has important theoretical and practical implications. The behavior of this system in "antigen excess" is reminiscent of the classical observations on precipitating antibody systems; it is possible that an optimum ratio of antigen to antibody is just as important in inducing human allergic reactions as it is in producing specific precipitates. Here, comparison must end, since there is yet no available chemical explanation for the effect of excess antigen on the histamine-releasing mechanism. Furthermore, such release of histamine is related to the logarithm of antigen concentration (Fig. 1); accordingly, "antigen excess" in this system requires an overwhelming amount of antigen compared to that involved in precipitating systems.

The second pattern was made apparent when attempts were made to relate our findings to skin reactivity. The degree of skin sensitivity did not correlate with either amount or fraction of available histamine released (Fig. 2).

A relationship did appear, however, when skin reactions were correlated with the concentration of pollen at which maximum histamine release occurred (Fig. 3). This relationship was not highly significant, perhaps because the methods for evaluating skin reactivity were routine and admittedly crude. However, the figures obtained on statistical analysis were noteworthy enough that further study along this line might be worth while (coefficient of correlation 0.52, regression coefficient −1.3, assuming a relationship $Y = a + b \log x$).

The rate of histamine release, as charted in Fig. 4, was relatively slow. Excess amounts of free histamine are removed from the circulation very rapidly, usually within one to two minutes, as determined by C^{14}-labeled histamine studies in man,[11] so that it is not surprising that little alteration in blood histamine levels can usually be detected in allergic diseases, such as bronchial asthma.[12] Further studies of this reaction should be carried out to confirm its linearity, which suggests that it is mediated by an enzyme system. An alternative possibility exists: that complete histamine release occurs instantaneously from individual leukocytes, but that the occurrence of this release varies randomly from 0 to more than 30 minutes among the various cells. If this were true, a sigmoid curve should be obtained for Fig. 4; our data do not include the many points along the curve necessary to rule out this type of phenomenon.

SUMMARY

Blood from forty-one persons with atopic disease was incubated with various concentrations of specific antigen. The amount of histamine released into the plasma was measured chemically.

Histamine was released by concentrations of pollen extract as low as 0.01 mcg. protein nitrogen per liter of blood; more histamine was released by greater amounts of antigen until a maximum level was reached at antigen concentrations of from 4 to 20 mcg. P.N. per liter of blood.

Histamine release tended to be suppressed by high concentrations of antigen unless massive amounts were used (4,000 mcg. P.N. per liter of blood), at which point nonspecific release occurred.

The degree of skin reactivity of the subjects studied was related inversely to the antigen concentration producing maximal histamine release.

The rate of histamine release was apparently linear, maximum release being approached only after at least thirty minutes of incubation of blood with the specific antigen.

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CITRIC ACID SENSITIVITY IN RECURRENT ULCERATIVE (APHTHOUS) STOMATITIS

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TUFT[1] has reported on a patient with recurrent ulcerative stomatitis in whom a marked sensitivity to citric acid was demonstrated as one, if not the only, etiological factor in the recurrence of these lesions. Tuft suggests that allergy to this material might be responsible for lesions in other patients with this syndrome.

Our study was directed at evaluating the relationship of citric acid and several other materials (which were described by Tuft as implicated also by virtue of their citric acid content or chemical relationship) in terms of a group of patients with documented recurrent ulcerative (aphthous) stomatitis.

Our criteria for selection of patients to be included in this group were as follows: Recurrent oral aphthae, when observed at their inception (which is infrequent), are generally seen as vesicles. Usually, the lesions are recurring ulcers, commonly from 1 to 12 mm. in size but occasionally measuring 2 to 3 cm., appearing anywhere in the oral cavity. They are extremely sensitive and painful, and often they are covered by necrotic membranes or exudates. The ulcers are most often round or oval, occasionally with irregular peripheries, and surrounded by an area of inflammation. Single or multiple lesions may recur periodically. The ulcers usually heal in from ten to fourteen days, but they are soon followed by new lesions or crops of lesions. An important differential diagnostic criterion is the absence of dermatologic lesions relatable to the oral aphthae. The clinical picture of patients with recurrent ulcerative stomatitis does not include the genital or eye symptoms characteristic of Behçet's syndrome or the skin lesions of erythema multiforme exudativum or pemphigus.

METHODS

Ten patients, three men and seven women, were included in this study. One additional woman, who had previously been afflicted with these lesions but was now in a prolonged stage of remission, was studied. The regimen followed with each subject was as follows: The patient was seated in a dental chair with a saliva ejector in place. Cotton rolls were used to remove obvious excess moisture from the entire mucobuccal fold area in the lower jaw.

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Crystals of either citric acid, potassium citrate, or ascorbic acid were placed in one of three separate areas while a swab saturated with acetic acid (vinegar) was placed in a fourth area of the mucobuccal fold. The areas of application were distributed throughout the mouth so that no area made contact with any other area. The swab impregnated with acetic acid (vinegar) was routinely placed in the second molar area on the right side, the citric acid in the lower right canine fossa, the ascorbic acid in the canine fossa on the left side, and the vitamin C crystals in relation to the lower second molar in the mucobuccal fold area on the left side.

These test materials were allowed to remain for five minutes, at the end of which time the patient rinsed the mouth thoroughly to remove all traces of these substances. The patient was questioned with regard to any sensations experienced during the above-described period, and observation was made immediately for evidence of contact erythema or allergy. The patient was then requested to sit quietly for fifteen minutes, following which time the areas were again "read" for evidence of inflammation or ulcer formation.

According to Tuft,[1] in his maximally sensitive patient irritation could be observed at the end of fifteen seconds and was well advanced after a period of five minutes. Our patients were dismissed after fifteen minutes but were requested to return twenty-four hours later and also seven days later. In numerous instances the patient was advised to look into his or her own mouth on the following day (because of the prolonged distances traveled by some of the patients in order to undergo this test) to ascertain any evidence of increased redness or ulcer formation. This was considered not unreasonable in such patients since, according to Tuft, an obvious typical ulceration occurred in the area of application of citric acid and also since these patients had become fully aware of the clinical picture of their lesions at all stages of development and regression after years of having lived with the aphthae.

RESULTS

No evidence of erythema was noticeable in any patient following the application of any of the four test substances at the end of the five-minute or fifteen-minute study periods. Six of the patients were examined after twenty-four hours without any evidence of erythema or other evidence of ulceration in any area. The remaining patients reported to us, by telephone or mail, that there had been no evidence of altered sensation or of a typical or atypical lesion in the area of contact testing. No lesions or other evidence of recurrent ulcerative stomatitis were in evidence in the particular areas tested, as seen by us or as reported to us by the patients after seven days.

It is our conclusion, therefore, that allergy of a contact nature to citric acid or any of the other related compounds tested was not an etiological factor in the lesions under consideration in our group of ten patients, although we fully accept the evidence offered by Tuft of maximal sensitivity of his patient

TABLE I

PATIENT	DRUGS APPLIED	OBSERVATIONS			
		5 MINUTES	15 MINUTES	1 DAY	1 WEEK
M. B.	Acetic acid (vinegar)	O	O	O	O
	Citric acid	O	O	O	O
	Na citrate	O	O	O	O
	Ascorbic acid	O	O	O	O
M. P.	Acetic acid (vinegar)	O	O	O	OM
	Citric acid	O	O	O	O
	Na citrate	O	O	O	O
	Ascorbic acid	O	O	O	O
M. T.	Acetic acid (vinegar)	O	O	OP	O
	Citric acid	O	O	O	O
	Na citrate	O	O	O	O
	Ascorbic acid	O	O	O	O
M. C.	Acetic acid (vinegar)	O	O	O	OP
	Citric acid	O	O	O	O
	Na citrate	O	O	O	O
	Ascorbic acid	O	O	O	O
P. O.	Acetic acid (vinegar)	O	O	OM	OM
	Citric acid	O	O	O	O
	Na citrate	O	O	O	O
	Ascorbic acid	O	O	O	O
E. M.	Acetic acid (vinegar)	O	O	O	O
	Citric acid	O	O	O	O
	Na citrate	O	O	O	O
	Ascorbic acid	O	O	O	O
L. T.	Acetic acid (vinegar)	O	O	OP	O
	Citric acid	O	O	O	O
	Na citrate	O	O	O	O
	Ascorbic acid	O	O	O	O
E. T.	Acetic acid (vinegar)	O	O	O	O
	Citric acid	O	O	O	O
	Na citrate	O	O	O	O
	Ascorbic acid	O	O	O	O
A. B.	Acetic acid (vinegar)	O	O	O	O
	Citric acid	O	O	O	O
	Na citrate	O	O	O	O
	Ascorbic acid	O	O	O	O
P. C.	Acetic acid (vinegar)	O	O	OP	OP
	Citric acid	O	O	O	O
	Na citrate	O	O	O	O
	Ascorbic acid	O	O	O	O
J. S.	Acetic acid (vinegar)	O	O	OP	OP
	Citric acid	O	O	O	O
	Na citrate	O	O	O	O
	Ascorbic acid	O	O	O	O

O = No visible change.
P = Reported by telephone.
M = Reported by mail.

to citric acid. We have concluded, however, that citric acid was a factor in that isolated patient only and should not be considered a routinely operating precipitating factor in recurrent ulcerative stomatitis.

The authors express appreciation to Mrs. Florence Moore for her assistance in coordinating this study and to Mrs. Eileen Geller and Mrs. Helene W. Kutscher for their assistance.

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RECURRENT ULCERATIVE (APHTHOUS) STOMATITIS: INTRADERMAL FOOD TEST STUDIES

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PREVIOUS investigations into the etiology of recurrent ulcerative (aphthous) stomatitis have been characterized by negative findings and failure. Reports describing successful therapy in this disease have been relatively unimpressive.

Recurrent oral aphthae, when observed at their inception (which is infrequent), are generally viewed as vesicles. Usually the lesions are seen as recurring ulcers, commonly from 1 to 12 mm. in size but occasionally measuring 2 to 3 cm., appearing anywhere in the oral cavity. They are extremely sensitive and painful and are often covered by necrotic membranes or exudates. The ulcers are usually round or oval, occasionally with irregular peripheries, and they are surrounded by an area of inflammation. Single or multiple lesions may recur periodically. The ulcers usually heal in from ten to fourteen days, but they are followed by new lesions or crops of lesions after variable remission periods. An important differential diagnostic criterion is the absence of dermatologic lesions relatable to the oral aphthae. The clinical picture of patients with recurrent ulcerative stomatitis does not include the genital or eye symptoms characteristic of Behçet's syndrome or the skin lesions of erythema multiforme exudativum or pemphigus.

Scattered through the literature, however, there have been numerous reports and comments implicating allergy, particularly allergy to foods,[6, 7] in the etiology of recurrent ulcerative (aphthous) stomatitis. According to Rowe,[6] allergic reactions are not infrequently seen in the mouth, throat, esophagus, stomach, intestines, rectum, and anus, depending upon the area which has been specifically sensitized previously. There may be more than one manifestation of allergy in the same patient.[6] Gastrointestinal symptoms, cheilitis, perlèche, angioneurotic edema, recurrent aphthae, stomatitis, gingivitis, and glossitis may possibly arise from food or drug allergy.[6] In our own experience, some patients have themselves suspected a food allergy as the cause but have been unable specifically to associate the onset with the ingestion of any particular food.

Despite the suggestions of an allergic etiology, however, no serious effort to test the thesis that recurrent ulcerative stomatitis results from food allergy has thus far been reported. In this study the possible allergic (to foods)

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etiology of recurrent ulcerative stomatitis has been investigated by means of intradermal food tests. It was hoped that it might also be possible to delineate subdivisions within the over-all classification of recurrent ulcerative (aphthous) stomatitis on the basis of those patients who represented a possible food allergy etiology and those who did not.

METHODS

Accordingly, thirteen patients aged 12 to 50 years, twelve females and one male, all presenting with recurrent ulcerative stomatitis, were studied by means of intradermal food tests in the allergy clinic of the Presbyterian Hospital.

TABLE I. SCHEDULE OF INTRADERMAL FOOD TESTS

FIRST DAY	SECOND DAY	THIRD DAY
Codfish	Corn	Dust 1/1.000
Clam	Rye	Orris 1/100
Beef	Oats	Kapok 1/100
Chicken	Lamb	Cotton seed 1/100
Pork	Flounder	Feathers 1/1.000
Milk	Walnut	Flaxseed 1/100
Green peas	Almond	Timothy 1/100
Lima beans	String beans	Ragweed 1/100
Tomatoes	Cauliflower	Tobacco 1/1.000
Orange	Beets	Alternaria 1/1.000
Mustard	Carrots	Hormodendrum 1/1.000
Wheat	Onions	Pyrethrum 1/1.000
Rice	Spinach	Dog 1/100
Potatoes	Asparagus	Wool 1/1.000
Banana	Celery	Horse 1/100
Chocolate	Pepper	Rabbit 1/100
Peanuts	Coffee	Cat 1/100
Egg	Apple	Silk 1/1.000
	Cantaloupe	
	Peach	
	Strawberry	

Eighteen food tests were administered the first day, twenty-one food tests on the second day, and eighteen inhalant tests (to selected patients) on the third day (Table I). Intradermal testing procedures were carried out by a standard procedure, during the course of which approximately 0.03 c.c. of an extract of the various foods, etc., was injected intradermally. Tests were read approximately twenty minutes therafter and were recorded as 0 to 4 plus.

RESULTS

The findings obtained for the thirteen patients with recurrent ulcerative stomatitis subjected to intradermal food testing procedures appear in Table II.

No striking pattern of food sensitivity could be established from systematic consideration of the tabulated results of our food-testing procedures.

These findings, interpreted in relation to results in normal patients (without allergy requiring diagnosis or therapy and specifically without recurrent ulcerative stomatitis), suggest that patients with recurrent ulcerative stomatitis demonstrate no allergy patterns not observable in a group of "normal" patients.

TABLE II. FINDINGS IN THIRTEEN PATIENTS WITH RECURRENT ULCERATIVE STOMATITIS SUBJECTED TO INTRADERMAL FOOD-TESTING PROCEDURES

PATIENT NO.	1	2	3	4	5	6	7	8	9	10	11	12	13	TOTAL NEGATIVE	TOTAL +−	TOTAL FAINT	TOTAL POSITIVE	OTHER
Egg		−		F	+	−	−	−	−	−	−	+	−	8	0	1	2	
Milk		−		−	F	−	−	−	−	−	−	F	−	9	0	2	0	
Wheat		−		−	F	−	−	−	−	−	−	−	−	10	0	1	0	
Codfish		−		−	+	−	−	−		−	−	−	−	9	0	0	1	
Green pea		−		−	F	−	−	−		−	−	−	−	9	0	1	0	
Rice		−		−	−	−	−	−	−	−	−	−	−	11	0	0	0	
Clam		−		−	−	−	−	−	−	−	F	−	−	10	0	1	0	
Lima bean		−	−	−	−	−	−	−	−	F	−	F	−	9	0	2	0	
Potato		−		−	−	−	−	−	−	−	−	−	−	11	0	0	0	
Beef		−		−	F	−	−	−	−	−	−	F	−	9	0	2	0	
Tomato		F		−	F	−	−	−	−	−	−	−	−	9	0	2	0	
Banana		−		−	F	−	−	−	−	−	−	−	−	10	0	1	0	
Chicken		F		−	−	−	−	−	−	−	−	−	−	10	0	1	0	
Orange		−		−	−	+	−	−	−	−	−	−	−	10	0	0	1	
Chocolate		+		−	+	−	−	−	−	−	−	−	−	9	0	0	2	
Pork		−		−	F	−	F	−	F	−	−	−	−	8	0	3	0	
Mustard		+		−	−	−	−	−	−	−	−	−	−	10	0	0	1	
Peanut		−		−	−	−	−	−	−	−	−	−	−	11	0	0	0	
Corn	−		−	−	+−	−	+−	+−	−	−	−	−	−	9	3	0	0	
String bean	+−		−	−	−	−	−	−	−	−	−	−	−	11	1	0	0	
Celery	−		−	−	+	−	−	−	−	−	−	+−	−	10	1	0	1	
Rye	−		−	−	−	−	−	−	−	−	−	−	−	12	0	0	0	
Beet	+		−	−	−	−	−	+	−	−	−	+	−	9	0	0	3	
Pepper	−		−	−	++	++	+	+	−	++	−	−	−	7	0	0	5	
Oats	−		−	−	−	−	−	−	−	−	−	−	−	12	0	0	0	
Cauliflower	−		F	−	−	−	−	−	−	−	−	+−	+	9	1	1	1	
Coffee	−		−	−	−	−	−	−	−	−	−	−	−	12	0	0	0	
Lamb	−		−	−	+	−	−	−	−	−	−	−	−	11	1	0	0	
Onion	−		−	+−	+	+	−	−	−	+−	+	+	−	6	2	0	4	
Apple	−		−	−	+−	−	+	−	−	−	−	−	−	10	1	0	1	
Flounder	−		−	−	−	−	−	−	−	−	−	−	−	12	0	0	0	
Carrot	−		−	−	+	−	−	−	−	−	−	−	+	10	0	0	2	
Cantaloupe	−		−	−	−	−	−	−	−	−	−	−	−	12	0	0	0	
Walnut	−		−	−	−	−	−	−	−	−	−	−	−	12	0	0	0	
Spinach	−		−	−	+−	−	−	+	−	−	−	−	F	9	1	1	1	
Peach	−		−	−	−	−	−	−	−	−	−	−	−	12	0	0	0	
Almond	−		−	−	−	−	−	−	−	−	−	−	−	12	0	0	0	
Asparagus	−		−	−	−	−	−	−	−	−	−	−	−	12	0	0	0	
Strawberry	−		−	−	+−	−	−	−	−	−	−	−	−	11	1	0	0	
Dust 1/1.000			−		−	F	−							3	0	1	0	
Timothy 1/100			−		−	−	−							4	0	0	0	
Dog 1/100			−		−	−	−							4	0	0	0	
Orris 1/100			−		−	−	F							3	0	1	0	
Ragweed 1/100			−		−	−	−							4	0	0	0	
Wool 1/1.000			−		−	−	−							4	0	0	0	
Kapok 1/100			−		−	−	−							4	0	0	0	
Tobacco 1/1.000			−		−	−	−							4	0	0	0	
Horse 1/100			−		−	−	−							4	0	0	0	
Cottonseed 1/100			−		−	−	−							4	0	0	0	
Alternaria 1/1.000			−		−	F	−							3	0	1	0	
Rabbit 1/100			−		−	−	−							4	0	0	0	
Feathers 1/1.000			−		−	−	−							4	0	0	0	
Hormodendrum 1/1.000			+−		−	−	−							3	1	0	0	
Cat 1/100			−		−	−	−							4	0	0	0	
Flaxseed 1/100			−		−	−	−							4	0	0	0	
Pyrethrum 1/1.000			−		−	−	−							4	0	0	0	
Silk 1/1.000			−		−	−	−							4	0	0	0	

Key: + = Positive. − = Negative. F = Faint.

DISCUSSION

The results of treatment of recurrent ulcerative (aphthous) stomatitis with antihistaminic agents (tripelennamine and Thenylene) have previously been reported as unsatisfactory,[2] although some amelioration of symptoms seemed to occur in some cases. Such findings would not be considered inconsistent with the effect of antihistaminics in cases of frequent exposure to allergens in patients with maximum allergy. In the present study, however, no patient with recurrent ulcerative stomatitis was found to be maximally sensitive to any food.

Before allergy to food can be excluded as an etiological factor, elimination diet therapy must be attempted, since it is acknowledged that skin tests may be negative to allergenic foods and that intradermal tests frequently may give false positive reactions which are not associated with clinical allergy. Study of patients by intradermal food testing was considered a necessary step toward eventual study by test-positive or elimination diet.

SUMMARY

Thirteen patients with prolonged history of recurrent ulcerative stomatitis were studied for food sensitivity by intradermal testing procedures. The pattern of skin sensitivity reactions elicited did not establish allergy to food as etiologically responsible for recurrent ulcerative stomatitis, in terms of the group as a whole or for individual persons therein, with regard to the group of foods as a *whole* or any food in particular.

The authors express their appreciation to Miss Lilian Vahey, R.N., and Mrs. Alice O'Connell, R.N., Mrs. Helene W. Kutscher, and Mrs. Florence Moore for their assistance in coordinating this study.

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IMMUNE RESPONSES TO RAGWEED POLLEN ANTIGENS

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INTRODUCTION

STUDIES with ragweed pollen, utilizing such analytic techniques as chemical fractionation,[1-3] ultracentrifuge,[4] electrophoresis,[5-9] chromatography,[10-13] ion exchange resins,[14] and serologic responses to experimental immunization,[15-16] have indicated the presence of multiple and distinct fractions within the pollen grain. Comprehensive reviews of this subject have been presented recently by Wodehouse[16] and Augustin.[17] The biologic activity of some of these pollen fractions, determined by their ability to elicit positive cutaneous test reactions of reaginic sensitivity in human beings, has been demonstrated. However, their exact roles as antigens and immunologic participants in the production of clinical manifestations of ragweed pollen allergy are still poorly understood.

Among other unanswered questions related to the problem of pollen sensitivity is the highly variable clinical response of patients treated with pollen extracts in attempts at hyposensitization. The value of ascertaining and understanding possible relationships between these two factors—that is, the biologic activity and the antigenicity of individual pollen constituents present in extracts available for treatment purposes—is self-evident. It is possible that in some patients one of the reasons for a lack of effective therapeutic response to specific pollen hyposensitization injections may be associated with a failure to incorporate the prerequisite qualitative, as well as quantitative, intracellular antigenic constituents of the intact pollen grain in treatment solutions. There has been no clear-cut demonstration that the current standard techniques of extraction result in soluble preparations which duplicate the antigenic pattern of their particulate pollen precursors.

An attempt to study this question has been one purpose of this report. This problem has been initially approached by attempting to study the immune response of experimental animals to ragweed pollen and derivative preparations. The antisera were employed in precipitin tests utilizing agar gel diffusion techniques to resolve each precipitin reaction into component antigen-antibody

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systems and to serve as an indicator of antigenic constituents of the ragweed preparations. This experimental approach is similar to that which we had previously employed in the study of bacterial intracellular antigens.[18]

The second purpose of this study has been concerned with an attempt to determine the ability of ragweed pollen to participate in those in vivo antigen-antibody reactions capable of inducing tissue manifestations of vascular sensitization. In this approach, the technique employed has been that of passive sensitization followed by subsequent challenge with specific antigen. This experimental procedure has been previously described in a demonstration that lesions of pulmonary periarteritis and panarteritis in experimental animals can arise from induced hypersensitivity reactions to foreign sera[19] and to bacteria or their metabolic products.[20-21]

EXPERIMENTAL METHODS AND RESULTS

Ragweed Antigens and the Production of Immune Sera.—Series of adult male rabbits were each immunized with one of the ragweed preparations described below, which was derived from the pollen of ragweed (*Ambrosia elatior*).

Each animal received a total dosage of 15 mg. protein nitrogen.* All injections by intravenous, intraperitoneal, or subcutaneous routes were given in fifteen equally divided doses over a twenty-one-day period, and the antiserum was prepared from blood drawn by cardiac puncture seven days after the last injection. In the case of intramuscular injections, the total dosage of ragweed antigen was adsorbed on aluminum hydroxide cream adjuvant[22] and administered in multiple sites in the gluteal areas on the same day, and antiserum was prepared from blood drawn by cardiac puncture twenty-one days later.

Immunizing ragweed antigens:

1. *Pollen hydrochloride* (ball-mill-ground whole pollen that had been first converted to pollen hydrochloride† according to the method of Rockwell[23]). This preparation was administered either by the intramuscular route or by a series of alternating intravenous and subcutaneous injections.

2. *Pollen cytoplasm* (solutions and suspensions of fragmented pollen grains that had been subjected to the disintegrating effects of either sonic waves at 10,000 cycles per second or ultrasonic waves at 800,000 cycles per second‡). This resulted in a disruption of the ectine and cell walls and subsequent release of intracellular pollen cytoplasm. This preparation was administered by two different techniques: (a) adsorbed on aluminum hydroxide cream and given by intramuscular injections and (b) a series of alternating intravenous and subcutaneous injections.

3. *Pollen extracts* (conventional saline extracts of ragweed pollen prepared by accepted methods of extraction, utilizing alkaline saline extracting

*Nitrogen determinations on all extracts and materials employed in this study were done at Center Laboratories, Port Washington, New York, through the courtesy of J. George Center.

†Delapan, supplied by Hollister-Stier Laboratories, Spokane, Washington, through the courtesy of Dr. E. L. Foubert, Jr.

‡Ultrasonic wave treatment of pollen was conducted through the courtesy of Dr. John M. Ward, Department of Biology, Temple University, Philadelphia, Pennsylvania, and under his direction.

fluid [Coca]). Extracts employing the following variations in preparation were utilized: (a) preliminary defatting of pollen with ether and (b) raw, nondefatted pollen; (c) addition of glycerin to extracting fluid; and (d) plain nonglycerinated. These extracts were administered to different series of animals by (a) intramuscular injection after adsorption of the extract on aluminum hydroxide cream or (b) a series of alternating intravenous and subcutaneous injections.

4. *Whole pollen grains* (a suspension of whole pollen grains, in an amount calculated to contain 15 mg. protein nitrogen, suspended in aluminum hydroxide cream and administered by intramuscular injections).

5. *Whole pollen subjected to multiple extraction.* Whole pollen grains, after thorough defatting treatment with ether, were subjected to repeated suspensions in alkaline saline extracting fluid (Coca) two to four times weekly. Following each extraction the supernatant fluid was tested for protein content and discarded, and the residual pollen was resuspended in a fresh supply of extracting fluid. This procedure was repeated over a period of two months until the supernatant fluid gave only a faintly positive test and the pollen mass had been reduced to one-fifth its original weight. This residual pollen, in an amount equivalent in weight to that used in whole pollen grains, was suspended in aluminum hydroxide cream and given by intramuscular injections.

6. *Pollen oil.* After treatment of the pollen with ether, the ether-soluble portion was filtered off, the ether was allowed to evaporate, and the residual pollen oil was added to liquid Albolene. Each rabbit received a total volume of 15 ml. of the oil-Albolene mixture prepared to contain that amount of pollen oil recovered from 15 grams of whole pollen grains. This was administered in fifteen equally divided doses by a series of alternating intraperitoneal and subcutaneous injections. Serum was prepared from blood drawn by cardiac puncture fourteen days after the last injection. The development of cutaneous sensitivity to pollen oil was determined by the appearance of an area of cutaneous erythema twenty-four to forty-eight hours following the application of the pollen oil in acetone to a shaved area of skin on the back or flank.

TABLE I. NUMBER OF BANDS OF PRECIPITATE FORMED IN THE INTERACTION OF RABBIT ANTISERUM TO RAGWEED POLLEN GRAINS AND TO DERIVATIVE PREPARATIONS WITH SOLUBLE RAGWEED POLLEN EXTRACTS AND CYTOPLASM IN AGAR GEL DIFFUSION PLATES (OUCHTERLONY TECHNIQUE)

	RAGWEED ANTIGENS		
RABBIT RAGWEED ANTISERUM TO:	EXTRACT OF RAW POLLEN GRAINS	EXTRACT OF ETHER-TREATED POLLEN GRAINS	SOLUBLE GRAIN CYTOPLASM (ULTRASONIC PREPARATION)
Ground pollen hydrochloride (Delapan)	1-2	1-2	1-2
Whole pollen grains	6	4-6	3-4
Saline extract and glycerine	4	3-4	2-3
Saline extract	2-3	2-3	1-3
Soluble cytoplasm (ultrasonic preparation)	1-2	2	1-2
Whole pollen grain defatted and hypoproteinated	3	2-3	2-3
Ether-soluble pollen oil	1	1	1

Precipitin Tests.—Tests for the detection of precipitin reactions between ragweed antigens and antisera to ragweed were carried out in agar-filled Petri dishes according to the diffusion technique described by Ouchterlony.[24] The soluble ragweed preparations and the antisera were placed in oppositely located wells within the agar to allow for their reaction while diffusing through the gel. Since each band of precipitate represented an independent antigen-antibody system, these precipitin reactions permitted the resolution of reactants into individual components. Double diffusion tests were employed to determine reactions of identity or nonidentity between the ragweed preparations for an evaluation of immunologic similarity or differences between their antigenic constituents.

The results of these precipitin tests are summarized in Table I.

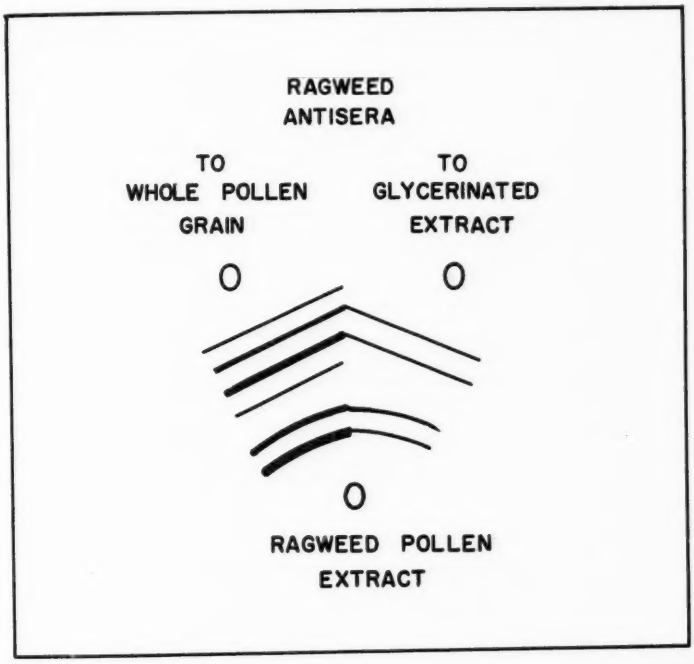

Fig. 1.—Diagrammatic reproduction of precipitin reactions occurring in an agar gel plate by the double diffusion technique of Ouchterlony employing tests with a saline ragweed pollen extract and two rabbit antisera: (1) to whole ragweed pollen grains and (2) to a glycerinated ragweed pollen extract.

In reactions of identity where bands of precipitate were common to antisera to both the whole pollen grain and pollen extracts or soluble pollen cytoplasm in equal amounts, the precipitin bands resulting from participation of the antiserum to the whole pollen grain were much heavier (often by five to ten times) than the corresponding common band (Fig. 1).

The antibody component of antiserum to the pollen oil was shared in common with antiserum to both the whole pollen grain and the pollen extract (Fig. 2).

Passive Sensitization.—Detailed discussions relative to procedure, interpretation, and description of the type of vascular lesions produced by the technique of passive sensitization and challenge employing foreign sera[19] and bacterial antigens[20-21] are reported elsewhere. Briefly, this consists of the administration of high-titer homologous species antiserum into the marginal ear vein of a rabbit. After a thirty-minute incubation period allowing for circulation, distribution, and fixation of the antibodies, the animal is challenged with the intravenous administration of a previously determined subanaphylactic dose of

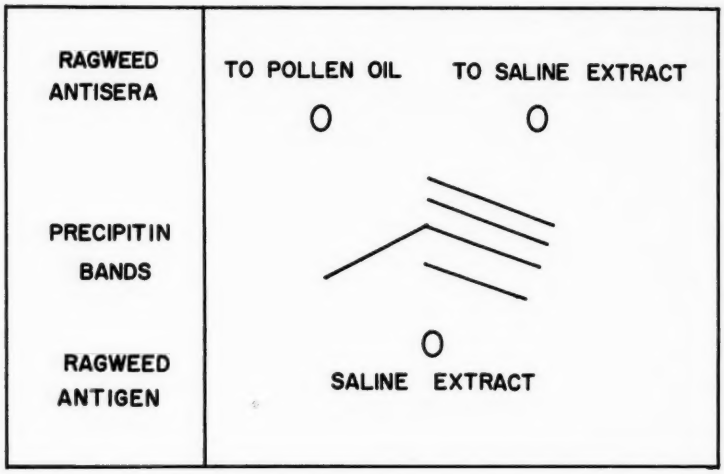

Fig. 2.—Diagrammatic reproduction of precipitin reactions occurring in an agar gel plate by the double diffusion technique of Ouchterlony employing tests with a saline pollen extract and two rabbit antisera: (1) to the ether-soluble fraction of ragweed pollen grains ("pollen oil") and (2) to a saline ragweed pollen extract.

specific antigen. This is divided into aliquots of 1/15, 2/15, 3/15, 4/15, and 5/15 of the total dosage, and each dose is administered at fifteen-minute intervals. Twenty-four hours later the animals are sacrificed by air embolism, and sections of internal organs are taken for study. The most constant lesion uniformly found is that of a pulmonary arteritis (described below). In the present study we attempted to extend this technique to include passive immunization with the rabbit antisera to ragweed pollen and subsequent challenge with ragweed pollen antigens whose methods of production and properties have been described previously in this report. All animals were male albino rabbits of the New Zealand strain, ranging in weight from 1.5 to 2.5 Kg.

Series 1 (Experimental Group): Six rabbits received 5 ml. each of a high-titer antiserum to ragweed pollen and were subsequently challenged with the intravenous administration of a total dosage of 0.15 mg. protein nitrogen ragweed pollen saline extract according to the procedure described above. In five of these six rabbits, hematoxylin-and-eosin-stained sections of the lungs studied microscopically revealed the typical lesions of periarteritis and panarteritis (Fig. 3). The small pulmonary arteries and the pulmonary arterioles showed marked

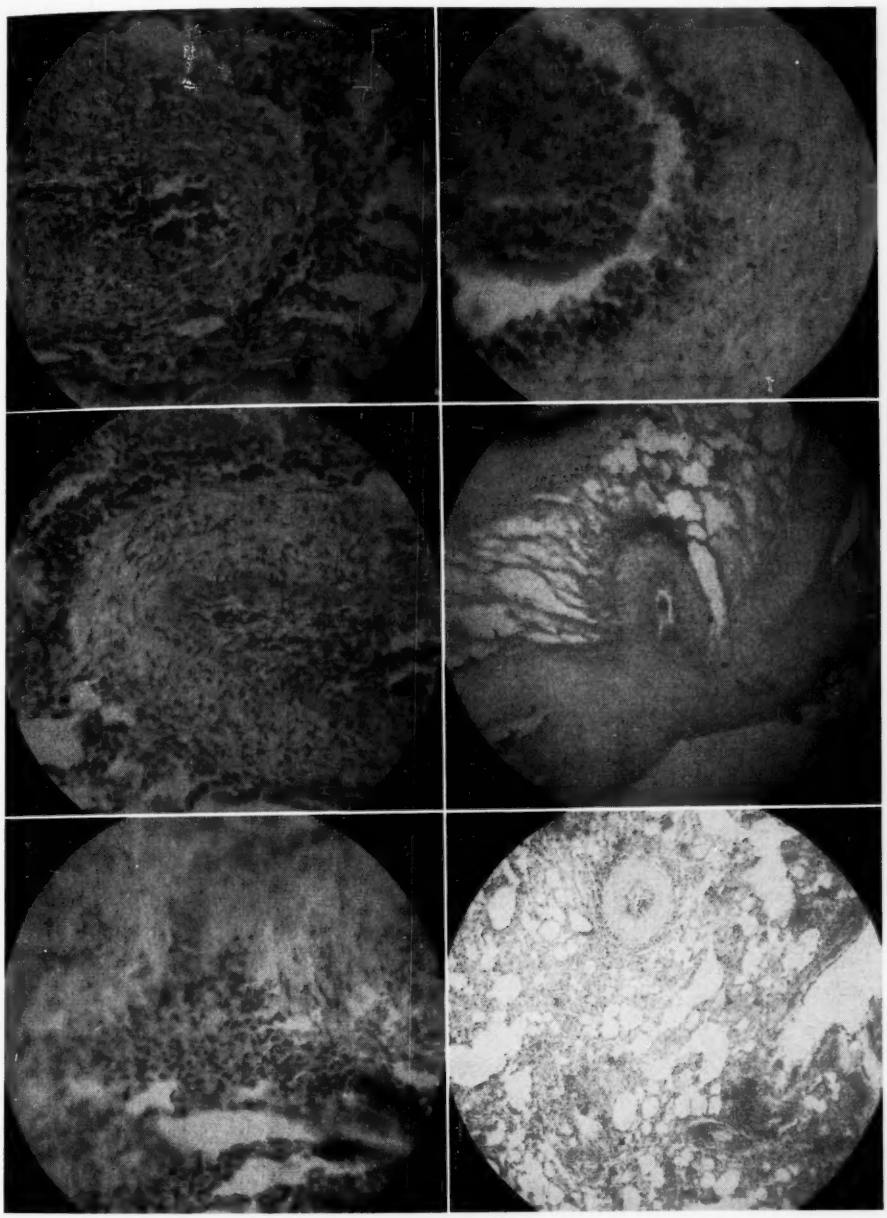

Fig. 3.—Sections from lungs of rabbits that had been passively sensitized with homologous species antiserum to ragweed pollen and subsequently challenged with soluble ragweed extracts twenty-four hours previously. These show involvement of pulmonary arteries and arterioles in varying degrees of arteritis. *A* and *B* show high-power magnifications of adventitial edema and cellular infiltration with eosinophils predominating and, to a very much lesser extent, with polymorphonuclear leukocytes and an occasional mononuclear cell. In *A*, *B*, and *D* there is involvement of the intima with edema, some early intimal cell proliferation, and infiltration with eosinophilic granular cells. Collections of eosinophils are seen within the lumen of the vessels shown in *A* and *B*, and in *D* there is an early thrombus formation containing eosinophils. In the lower left field of the vessel shown in *A* there is disruption in the integrity of the internal elastic membrane. A nodular collection of eosinophils is seen within the adventitia and penetrating into the media of the vessel seen in *C*. Low-power magnification shows a mild perivascular ringing with eosinophils and an intimal reaction of the arteriole shown in *E*. At the upper pole of the branching vessel shown in *F* there is a diffuse nodular collecton of eosinophils in the adventitia and extending into the adjacent alveolar tissue.

perivascular ringing and adventitial infiltration, especially of eosinophils, with few polymorphonuclear leukocytes and only an occasional lymphocyte and mononuclear cell. Eosinophils were also found infiltrating the media and intima of the arteries, and in several instances collections of eosinophils were found within the lumina of these vessels. Varying degrees of hyperplasia of the endothelial cells of the intima and adventitial edema were also found.

Series 2 (Control Group A): Six rabbits each received only 5 ml. of antiserum to ragweed pollen by the intravenous route, and in none of these animals could microscopic pathologic lesions of the vasculature be demonstrated.

Series 3 (Control Group B): Each of six rabbits received only ragweed pollen saline extract intravenously in divided dosages totaling 0.15 mg. protein nitrogen. Microscopic pathologic vascular lesions were absent in all six of these animals.

DISCUSSION

The nature of some immunologic factors associated with the precipitation of rabbit antisera to ragweed in specific in vitro antigen-antibody reactions has been studied by Bukantz and associates,[25] who applied quantitative precipitin techniques, and by Portnoy and Sherman,[26] who employed complement fixation procedures. Thus, it has been possible to utilize immune sera in evaluating such poorly understood points as the effect of storage and preservatives on ragweed extracts[25] and the inhibiting effects of the blocking antibody.[25, 27] Becker and Munoz[15] and Wodehouse[16] have employed rabbit antisera in agar gel diffusion precipitin techniques in attempting antigenic analysis of ragweed pollen extracts, and Wodehouse[28] further suggests their application in standardization of pollen extracts. Although these reports have established the validity of interpreting ragweed antigen-antibody precipitin reaction bands in an agar gel, the results of our studies tend to demonstrate the many variables inherent in the qualitative antibody components of rabbit antiragweed sera. These apparently are partially dependent upon the particular type and state of the ragweed preparation employed in immunization procedures. Differences were noted in the uniformity of test patterns resulting from the participation of antisera that had been produced against standard pollen extracts and the participation of antisera to the whole pollen grain. This points to the need for a clearer understanding and interpretation of the qualitative antigenic analysis of native ragweed pollen as a prerequisite for the use of animal precipitating antisera in pollen extract standardization.

The precipitinogenic activity of immune rabbit serum can lend itself well to a biologic function as an indicator of antigenic components of the ragweed pollen grain and derivative preparations. However, there is no attempt to compare the rabbit's immune response to ragweed pollen with those still poorly understood conditions associated with the state of natural reaginic hypersensitivity in man or with his reaction to induced measures of hyposensitization that result in the production of blocking serum antibodies. Wodehouse[16] feels that

some of the antigens represented by precipitation bands have been identified with a fair degree of certainty as those biologically active antigens revealed by chemical fractionation, electrophoresis, and chromatography. Augustin[17] has noted that the experimental evidence furnished by clinical trial, skin reactivity of dissolved washed floccules, and correlation of skin tests with diffusion tests of extracts points to, but does not prove, the premise that the grass pollen allergens of man are the precipitin-producing antigens of the rabbit.

The exact number of biologically active fractions within the ragweed pollen grain has not been uniformly determined, and results of fractionation procedures have varied with methods employed. The total number of fractions reported has varied from two or three, as first determined by chemical fractionation,[1] upward to eleven or twelve with the employment of newer techniques utilizing ion exchange resins[14] and chromatography.[10-11] There is insufficient evidence, however, to indicate that all these fractions are antigenic or biologically active. The finding, in the whole pollen grain, of six components, sufficiently antigenic to give rise to individual rabbit serum precipitins roughly parallel the findings of Rockwell,[3] who isolated five fractions by chemical fractionation; Abrahamson and associates,[5, 8] who reported the presence of one major and six or more minor fractions in studies employing electrophoresis and the ultracentrifuge; Dankner and his co-workers,[12] who employed paper chromatography and reported six and seven active components; and also those of Becker and Munoz,[15] who noted five antigenic fractions capable of precipitinogenic action with immune rabbit sera in a gel diffusion system with the Oudin technique. Although we employed the same method of precipitin testing as Wodehouse,[16] we were unable in any instance to duplicate his finding of at least eight component antigens in short ragweed pollen extract. Cebra's[29] explanation would assume that, because of overlapping of bands and unfavorable concentration of reactants, a submaximal number of bands will be revealed by complex testing mixtures in tests with ragweed extracts containing as many as ten antigens.

The discrepancy in the results of these studies may, of course, be related to the actual technical methods employed. However, other explanations considered include those reports that (1) electrophoretic analysis demonstrated that these fractions may be mixtures[6] and that some contain common and independent allergens,[30] (2) proteins in ragweed extracts gradually break down into fragments of lesser magnitude,[7] and (3) these fractions show extreme lability which results in fragmentation.[12-13] In this regard, our present findings of distinct variations in qualitative antibody content of immune rabbit sera directly related to the type of immunizing ragweed antigen employed would tend to suggest that the mode of preparation of a ragweed extract may affect its qualitative antigenic content.

It is possible that the intact pollen grain may contain more than the maximum number of six detected constituent antigens, intracellularly or within the cell wall. This has been impossible to determine, however, because technical limitations imposed by the technique of gel diffusion preclude the use of any

but soluble antigens. In converting the whole pollen grain to a soluble extract there apparently occurred in at least two of the components a loss in antigenicity sufficient to effect their immunizing potential. The relatively greater antibody content in antiserum to the whole pollen grain over that of antiserum to soluble extracts suggests the antigenic superiority characteristic of most cellular or particulate antigens.[31] This is similar to the finding of greater antibody content in antiserum to bacterial cells in comparison with antiserum resulting from immunization with soluble bacterial antigens.[18]

It was felt that perhaps the advantages of the enhanced immune response to the native intact pollen grain might be simulated with a more feasible immunization technique utilizing soluble pollen protoplasm. An attempt was then made to recover such cytoplasm by disrupting the integrity of the ectine and cell walls of the intact grain by subjecting pollen to the disintegrating effects of ultrasonic and supersonic vibrations. However, modifications in the integrity of the whole pollen grain by physical factors, whether ultrasonic wave disruption or mechanical grinding, apparently resulted in antigenic alterations characterized by a loss of immune response to as many as four or five active pollen constituents. These effects appear to be similar to those noted following ball mill grinding and/or ultrasonic wave exposure to bacterial cells where antigenic modifications of soluble bacterial cytoplasm have also been demonstrated.[18] This appears to be generally true of most cellular antigens. Following traumatic procedures, different antigens may possibly arise as a result of further splitting and/or structural rearrangement or formation of new combinations of the individual constituents that originally composed the complex but labile molecular aggregates of the intact cell.[29]

Generally there was very little difference noted in the respective numbers of antigenic constituents of saline-extracted raw ragweed pollen and that prepared from pollen subjected to preliminary treatment with a fat solvent. Such defatting procedures may therefore result in only minor changes. In an occasional animal, however, a loss of immune response to one or two antigenic components did occur.

The addition of glycerin apparently enhanced the antigenic effect of saline extracts by increasing the qualitative immune response to one or two antigenic components of the pollen grain. It is not known whether this may be due to some adjuvant effect of glycerin or whether, as shown by Bukantz and coworkers,[25] nonglycerinated pollen extracts may deteriorate rapidly in biologic action under storage conditions.

It is interesting to note the extreme difficulty experienced in completely removing antigenic material from ragweed pollen, even after periods of as long as two months of multiple and repeated extractions with a resultant loss of three-fifths to four-fifths of the original weight of the pollen mass. Thus, much valuable ragweed antigen, both qualitative and quantitative, may remain within the whole pollen grain after standard extracting procedures. Rockwell[23] had previously estimated that only one-sixth of the active antigen is extracted in the preparation of crude pollen extract.

The presence of circulating precipitating antibody to one major antigenic component of ragweed pollen in the sera of animals injected with the ether-soluble fraction of the pollen grain ("pollen oil") was a surprising finding, in view of the accepted concept of the selective action of proteins and carbohydrates as antigens in such immunologic phenomena. The presence of this serum antibody was not related to an associated development of cutaneous contact sensitivity to the ragweed oil. The fact that this particular antigenic constituent of the pollen oil is shared in common with soluble pollen cytoplasm and pollen extract suggests two possible explanations: (1) that this is really a water-soluble protein constituent taken down in colloidal suspension in an ether-water mixture because of inability to extract the pollen grains under completely anhydrous conditions and (2) that this constituent may be a lipoprotein soluble in ether saline or ether. However, we have been unable thus far to identify it as such by selective precipitin band staining with Sudan black B or oil red R.[32] The presence of this common antigen in both ragweed extract and pollen oil may help to explain the unusual finding of both the positive intracutaneous whealing reaction to pollen extract and the positive contact reaction to pollen oil by patch test technique in the same ragweed-sensitive patients.[33-34]

Thus, it is seen that current accepted methods of extraction in the preparation of ragweed antigens for clinical use in hyposensitization techniques may fall short of duplicating the antigenic pattern of the whole ragweed pollen grain. However, whether this is actually necessary for satisfactory treatment of the ragweed-sensitive patient, together with other undetermined points concerned with an exact identification of those precipitin bands to indicate pollen constituents whose presence is necessary to evoke such an immune response, is not known. If the immune response of an experimental animal is any indication of the antigenic efficacy of a ragweed extract required for human treatment purposes, then the search for feasible techniques for duplicating immunization with all native intracellular pollen antigenic fractions without antigenic alteration or denaturation should be the object of further investigation.

The microscopic finding of lesions of periarteritis and panarteritis of the pulmonary tissue in sensitized and challenged rabbits indicates the vascular sensitizing properties of ragweed pollen in this species. The experimental conditions associated with the production of these lesions suggests that the development of hypersensitivity vasculitis is dependent upon a shock tissue response to the specific interaction of antigen and antibody rather than a toxic action of the ragweed pollen antigens themselves or an in vivo effect of their antibodies per se. Histologic appearance of these vascular lesions and the immunologic prerequisites for their production simulated in detail the features associated with the experimental production of arterial sensitization observed with other antigen-antibody systems related to foreign sera,[19] bacteria, and bacterial products.[20-21] Thus, there is offered further evidence of the validity of our previous suggestion that the development of pulmonary arteritis in the rabbit may be a tissue manifestation of the subacute anaphylactic reaction and that it suggests the potential of ragweed pollen antigen for participation in hypersensitivity reactions affecting the vasculature.

SUMMARY

1. Series of rabbits were experimentally immunized with either intact grains of whole ragweed pollen or one of its derivative preparations, including saline extracts, soluble cytoplasm obtained by subjecting pollen grains to the disintegrating effects of ultrasonic and supersonic vibrations, ball-mill-ground whole pollen hydrochloride, and ether-soluble fraction ("pollen oil").

2. The antisera obtained from these animals were employed as indicators of antigenic components of ragweed pollen in determinations of patterns of precipitin test reactions employing agar gel diffusion plate techniques (Ouchterlony).

3. The interpretation of antigen-antibody precipitin band patterns suggests the following:

(a) The maximum number of antigenic components in ragweed pollen that could be determined by this technique was six.

(b) There is both qualitative and quantitative antigenic superiority in the whole ragweed pollen grain, which could not be duplicated with immunization procedures employing preparations of pollen extracts or soluble pollen cytoplasm.

(c) Conversion of the whole pollen grain to a soluble extract may result in an antigenic alteration involving as many as two pollen constituents.

(d) Soluble pollen cytoplasm obtained by disrupting the integrity of the ectine and cell walls of the pollen grain by mechanical procedures may undergo a loss of two or three antigenic components.

(e) Preliminary defatting of the pollen with ether results in only minor antigenic effects, but the addition of glycerin to a saline ragweed extract enhances its antigenicity.

(f) Ragweed pollen subjected to standard and prolonged extraction procedures contains effective residual antigenicity.

(g) Ragweed pollen oil contained one antigenic fraction of undetermined nature capable of inducing a serum precipitating antibody response.

4. In rabbits passively sensitized with homologous species antiserum to ragweed and subsequently challenged with specific soluble pollen extract, microscopic lesions of periarteritis and panarteritis of the pulmonary vasculature were demonstrated. These lesions could not be found in rabbits receiving either the antiserum or the pollen antigen alone. The development of hypersensitivity vasculitis as a tissue response to the in vivo ragweed pollen antigen-antibody reaction is suggested.

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American Academy of Allergy

REPORT OF THE COMMITTEE ON DRUGS OF THE RESEARCH COUNCIL OF THE AMERICAN ACADEMY OF ALLERGY, 1957-1958

THE Committee on Drugs has continued its activity on an intensive basis during the past year. Efforts to obtain new and promising drugs at an earlier stage of their investigation are being pursued. A number of worthwhile projects concerned with drug evaluation were submitted and undertaken for study. In addition, the group has also considered the study of certain agents whose effects are poorly understood. An example of this is the current long-term study with gamma globulin. The Committee also has been called upon to investigate unsubstantiated claims of treatment of allergic disease and to render opinions to the Food and Drug Administration in several areas of their activities. A more recent function is assistance to other groups of medical specialists in relation to specific allergy problems with which they are faced in their own specialties.

The present report covers projects which are completed or far enough along in their study to merit comment at this time.

CORTROPHIN-ZINC (ORGANON INC., ORANGE, NEW JERSEY)

Cortrophin-zine is Organon's brand of corticotropin-zinc hydroxide, each cubic centimeter of which contains 40 U.S.P. units of corticotropin combined with 2.0 mg. of zinc. It is a fine aqueous suspension requiring no preheating, and it flows freely through a 24-gauge needle. Plasma levels of 17, 21 dihydroxy-20-ketosteroids are reported to be significantly elevated for longer periods of time following the intramuscular injection of a single dose of 20 to 40 units of Cortrophin-Zinc as compared to ACTH gel. These levels are claimed to be comparable to those obtained following continuous intravenous administration of 20 to 25 units of corticotropin over an eight-hour period.[1]

The purpose of the Committee's study was to compare the effectiveness of the newer zinc preparation with ACTH gel in severe allergic states. The points of comparison which were felt worthy of exploration were the following: (1) clinical effectiveness in relieving symptoms; (2) dosage schedule required to suppress symptoms; (3) dosage schedule required to maintain improvement; (4) duration of action of a single dose; (5) ease of administration; and (6) local discomfort following administration.

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The following investigators contributed to the study: Theodore B. Bernstein, Evanston, Illinois; Joseph Besser, Philadelphia, Pennsylvania; Seymour Crepea, Madison, Wisconsin; Earl B. Brown, New York, New York; Harold Friedman, Cleveland, Ohio; Alex S. Friedlaender, Detroit, Michigan; Sidney Friedlaender, Detroit, Michigan; Bert Schoenkerman, Milwaukee, Wisconsin; and Aaron Spielman. New York, New York. Each participant was supplied with two preparations by the Organon Company: Cortrophin-Zinc and corticotropin gel, each containing 40 U.S.P. units of corticotropin per cubic centimeter. A total of eighty-four case reports were submitted for summary. These consisted of sixty-six patients with bronchial asthma, twelve with atopic dermatitis, four with urticaria and angioneurotic edema, and two with allergic ocular conditions.

Results.—In the entire group of eighty-four patients treated with Cortrophin-Zinc, results were rated as good to excellent (more than 50 per cent improvement) in sixty-five cases and poor to fair (less than 50 per cent improvement) in nineteen cases. Individual doses varied from 10 to 80 units, with total suppressive doses ranging widely from 30 to 500 units administered over periods of one to five days. These wide variations were necessarily dictated by the severity of each patient's condition, as well as the judgment of the individual investigator. Maintenance doses varied from 10 to 80 units given at intervals of twelve hours to seven days. The majority of patients were maintained on doses of 40 units every twenty-four to seventy-two hours.

TABLE I. COMPARISON OF CORTROPHIN-ZINC AND ACTH GEL IN FORTY-EIGHT PATIENTS WITH BRONCHIAL ASTHMA

	CORTROPHIN-ZINC BETTER	ACTH GEL BETTER	NO DIFFERENCE	
Clinical effectiveness	4	2	42	
Total dosage to suppress symptoms	4	2	42	
Dosage to maintain improvement	11	1	36	
Duration of action of single dose where determined	12	1	13 (same)	22 (Unable to Determine)
Ease of administration	42	0	6	

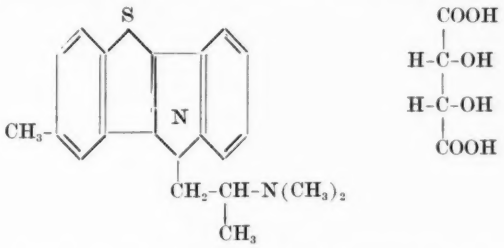
Since the primary purpose of the study was a comparison between the zinc and gel preparations, efforts were made to compare the two in the same patients. This was possible in forty-eight cases of bronchial asthma, and the results are summarized in Table I. The clinical effectiveness and total dosage necessary to suppress symptoms appeared to be equivalent for the two preparations. A lesser amount of Cortrophin-Zinc was required to maintain improvement in eleven patients, more was necessary in one patient, while in thirty-six patients the amount was the same. In twenty-six patients in whom it was possible to compare the duration of action from a single dose, the effect of Cortrophin-Zinc was longer than the gel in twelve instances, less in one, and the same in thirteen others. The ease of administration of the zinc preparation

was definitely greater than the gel, which requires warming prior to administration. However, local discomfort, consisting of soreness, redness, or induration, was reported more often with the zinc material than with the gel. Sixteen patients reported local reactions with Cortrophin-Zinc, nine complained of discomfort with both preparations, and the remainder experienced no difficulty. A more recent preparation of Cortrophin-Zinc containing 1.0 mg. of zinc hydroxide per cubic centimeter was subsequently tried by several of the participants with noticeable reduction in these local effects.

Comment.—A comparison of Cortrophin-Zinc and corticotropin gel indicates approximately equal therapeutic effectiveness for the two preparations in severe allergic states. While an increased duration of action from a single dose of Cortrophin-Zinc could not be uniformly demonstrated, some cases appeared to be maintained with less material given at longer intervals. The zinc preparation is considerably easier to administer than the gel, but it may produce more local discomfort.

ANTIHISTAMINIC SU-4173 (CIBA PHARMACEUTICAL PRODUCTS, INC., SUMMIT, NEW JERSEY)

Pharmacologic Background Data.[2]—SU-4173 (8-methyl-10 [2-dimethylaminopropyl] thianaphthindole tartrate) has the following formula:



The drug has shown a high potency and prolonged action when tested as an antagonist of histamine in the isolated guinea pig ileum. On oral administration in the intact animal, SU-4173 demonstrates a more intense and prolonged action against histamine than does Pyribenzamine. It is highly soluble, in both water and physiologic saline, which appears to enhance its absorption on oral administration. Toxicity is low, the intravenous LD_{50} being 58.3 ± 4.16 mg. per kilogram.

Clinical Studies.—Study of this drug was undertaken primarily because of pharmacologic evidence of high potency and prolonged effects against histamine, suggesting that less frequent administration of the drug might be effective in certain allergic states. To determine the duration of action, minimal dosage was therefore advised since it was hoped that a single dose every twelve hours might be sufficient. The final dosage requirement for each patient was determined by the individual investigator. It was supplied in the form of 100 mg. uncoated tablets in time for use during the 1957 seasonal pollen periods. The majority of cases treated were those of seasonal allergic rhinitis.

TABLE II. CLINICAL RESULTS WITH ANTIHISTAMINIC SU-4173 IN SEASONAL ALLERGIC RHINITIS

DAILY DOSE (NUMBER OF 100 MG. TABLETS)	TOTAL PATIENTS TREATED	MARKED RELIEF	MODERATE RELIEF	SLIGHT RELIEF	NONE
1	4	2 (50%)			2 (50%)
2	128	21 (16.4%)	58 (45.3%)	27 (4%)	22 (17.1%)
3	78	14 (17.9%)	34 (43.9%)	16 (20.5%)	14 (17.9%)
4	14	1 (7.1%)	3 (21.4%)	7 (50%)	3 (21.4%)
Totals	224	38 (16.9%)	95 (42.4%)	50 (22.3%)	41 (17.4%)

TABLE III. CLINICAL RESULTS WITH ANTIHISTAMINIC SU-4173 IN OTHER ALLERGIC CONDITIONS

DAILY DOSE (NUMBER OF 100 MG. TABLETS)	PERENNIAL ALLERGIC RHINITIS					ATOPIC DERMATITIS					URTICARIA AND ANGIONEUROTIC EDEMA				
		RELIEF					RELIEF					RELIEF			
	TOTAL CASES	MARKED	MODERATE	SLIGHT	NONE	TOTAL CASES	MARKED	MODERATE	SLIGHT	NONE	TOTAL CASES	MARKED	MODERATE	SLIGHT	NONE
2	10	1	6	2	1	1		1							
3	12	3	5	4	0	1		1			2	1	1		
4	6		2	1	3	1				1	2			2	
More than 4	4		1		3						1			1	0
Totals	32	4 (12.5%)	14 (43.7%)	7 (21.5%)	7 (21.5%)	3	0	2	0	1	5	1	1	3	

Case reports were contributed by the following investigators: Murray M. Albert, New York, New York; Earl B. Brown, New York, New York; Theodore B. Bernstein, Evanston, Illinois; Alex S. Friedlaender, Detroit, Michigan; Sidney Friedlaender, Detroit, Michigan; Harold Friedman, Cleveland, Ohio; Milton Mosko, Chicago, Illinois; Isadore Pitesky, Long Beach, California; Bert Schoenkerman, Milwaukee, Wisconsin; Sheppard Siegal, New York, New York; and Aaron Spielman, New York, New York.

A total of 264 patients were studied, consisting of 224 patients with seasonal allergic rhinitis, thirty-two with nonseasonal allergic rhinitis, three with atopic dermatitis, and five with urticaria and angioneurotic edema.

Results.—The over-all effectiveness of 100 mg. doses of SU-4173 did not differ markedly from that of standard doses of presently available active antihistaminic drugs. While the symptoms in some patients were controlled very well on one tablet every twelve hours, a substantial number required more frequent administration. Some did better when the daily dose was increased to three or four tablets daily. Others failed to achieve benefit at higher dosage levels and were frequently transferred to another drug. The results of treatment at various dosage levels are recorded in Tables II and III.

TABLE IV. DURATION OF RELIEF FROM SINGLE DOSE OF SU-4173 COMPARED TO OTHER ANTIHISTAMINICS USED

TOTAL PATIENTS COUNTED	LONGER	SHORTER	SAME
142	57 (40.1%)	34 (23.6%)	51 (35.9%)

In 142 patients, a comparison of the duration of action of a single dose of SU-4173 and the antihistaminic drug previously used by the patient showed that the new compound gave a longer period of relief in fifty-seven patients (40.1 per cent), a shorter period in thirty-four (10.3 per cent), and no noticeable difference in fifty-one (35.9 per cent) (Table IV).

TABLE V. SIDE EFFECTS (MARKED TO MODERATE) WITH ANTIHISTAMINE SU-4173

COMPLAINT	NUMBER OF PATIENTS	
Sedation	51	(19.3%)
Gastrointestinal	7	(2.6%)
Other		
Dryness of mucous membranes	3	
CNS stimulation	2	
Mental depression	1	(3.0%)
Palpitation	1	
Headache	1	
None	198	(75.0%)
Total patients treated	264	

Side Effects.—Sedation was the principal side effect, occurring to a marked or moderate degree in fifty-one patients (19.3 per cent). Gastrointestinal complaints were next in frequency, occurring in seven patients (2.6 per cent). Miscellaneous side actions were recorded in eight other patients (3.0 per cent) (Table V).

Comment.—The clinical results indicate that SU-4173 in 100 mg. doses is a generally well-tolerated, moderately active, antihistaminic agent. While a duration of action of eight to twelve hours from a single dose was apparent in some cases of allergic disease, the period of relief in a high percentage of cases was not greatly different from that experienced with standard doses of presently available active antihistaminic drugs.

GAMMA GLOBULIN STUDY

One year ago a study on the use of gamma globulin in asthma associated with infection was initiated by the Committee. Since a great deal concerning gamma globulin therapy is poorly understood, the intent of this project was to collect a statistically significant group of suitable cases for analysis. The Committee on Drugs was encouraged in their work by the American National Red Cross, who considered the study important enough to allot adequate supplies of gamma globulin through the Red Cross blood program. This project has also been of great interest to many members and Fellows of the Academy. Presently cooperating in the study are Clarence Bernstein, Orlando, Florida; Noah Barysh, New Milford, Connecticut; Joseph Besser, Philadelphia, Pennsylvania; Robert J. Becker, Joliet, Illinois; Harry Bernton, Washington, D. C.; Earl B. Brown, New York, New York; Seymour Crepea, Madison, Wisconsin; Leo H. Criep, Pittsburgh, Pennsylvania; Alex S. Friedlaender, Detroit, Michigan; Sidney Friedlaender, Detroit, Michigan; Samuel M. Feinberg, Chicago, Illinois; Edmund L. Keeney, La Jolla, California; Samuel Levin, Detroit, Michigan; Dean A. Moffat, Salt Lake City, Utah; John P. McGovern, Houston, Texas; Isadore Pitesky, Long Beach, California; Simon Rubin, Gary, Indiana; Aaron Spielman, New York, New York; and Edward L. Strem, St. Paul, Minnesota. Dr. Seymour Crepea, Madison, Wisconsin, has served as coordinator for the Committee in the task of accumulating and analyzing the data from the many sources involved. An interim report is presented at this time, since the Committee intends to continue this study for another year.

Participants in the study were asked to select suitable cases of asthma in which the element of bacterial infection was considered to be a highly important primary or secondary factor, and the patients were adequately studied from an allergic point of view. Only general recommendations were made regarding dosage and frequency of administration. It was suggested, however, that initially infants and children might be more suitable for study, since dosage on a weight basis could be adjusted to higher levels, until more definite information regarding dosage requirements was obtained. In other studies for the control of various infectious states, the dosage range of gamma globulin has extended up to 0.6 c.c. per kilogram of body weight. If such high amounts or higher should prove necessary in the present study, it would be more difficult to administer adequate doses of the presently available material to adult patients. However, a substantial number of adult subjects have been included in the study to date.

Investigators were also requested to obtain for each patient, before and at periods during treatment, complete blood counts, sedimentation times, serum

electrophoretic studies, or chemical determinations of gamma globulin where facilities for such were available.

Results.—Interval reports were obtained in eighty-four cases to date. Their ages ranged from 6 months to 63 years. Individual doses of gamma globulin on a weight basis varied from 0.024 c.c. to 0.66 c.c. per kilogram. The frequency of injections extended from once daily to once every month.

Presence or absence of low serum gamma globulin levels before treatment, and the dose of gamma globulin administered were considered the most significant points of interest in guiding our future course in this project. Twelve out of eighty-four patients showed hypogammaglobulinemia (Table VI). It was significant (27.7 per cent) only in the group up to 5 years of age, being noted in ten out of thirty-six patients. Improvement occurred in a greater percentage receiving doses of 0.30 c.c. per kilogram of body weight than below this level.

TABLE VI. GAMMA GLOBULIN THERAPY IN ASTHMA WITH INFECTION

AGE (YEARS)	NUMBER OF PATIENTS	REPORT OF LOW GAMMA GLOBULIN BEFORE TREATMENT
0- 5	36	10 (27.7%)
6-20	30	0
21-40	8	0
41 and up	10	2
Totals	84	12

Comment.—No conclusions are intended or warranted at this time, but it is felt that the results to date may be utilized as a guide to the establishment of more definite dosage recommendations in the continuation of this group study. While advice as to the most satisfactory dose and interval of gamma globulin administration cannot yet be given, it appears, from present data and from the work of others concerning the fate of injected gamma globulin in the body, that doses of at least 0.30 c.c. per kilogram of body weight or higher, administered at intervals of two to four weeks, would be practical in such therapy.

ANERGEX (MULFORD COLLOID LABORATORIES, PHILADELPHIA, PENNSYLVANIA)

Anergex was used by members of the Committee in twenty-five patients with allergic disease. Its use according to the method outlined by the manufacturer failed to show any striking therapeutic effect. Results submitted to the Committee by three independent investigators covering forty-four similar cases were also poor. Further study of the product by the Committee on Drugs did not appear warranted. A more detailed report covering the work with this product has already been circulated among the membership of the Academy in the form of a newsletter.[3]

PENICILLINASE ("NEUTRAPEN," SCHENLABS, NEW YORK, NEW YORK)

This preparation of injectable penicillinase has recently been submitted to the Committee for evaluation in the treatment of penicillin reactions. Clinical reports thus far attesting to its value in delayed urticarial reactions have

been very favorable.[4, 5] The manufacturer also asserts that the material may have some value in the immediate anaphylactic type of penicillin reactions. These claims are considered worthy of further exploration on a group basis, and the product was accepted for study.

OTHER ACTIVITIES

The Committee was asked to work with radiologists in the problem of severe reactions to radiopaque materials used in x-ray procedures. Preliminary meetings have been held with representatives of the American College of Radiologists and the Food and Drug Administration in connection with the efficacy and safety of present pretesting procedures and the prevention and treatment of allergic reactions from the use of such materials.

SUMMARY

Activities of the Committee on Drugs during the past year have included clinical studies with Cortrophin-Zinc (corticotropin-zinc hydroxide); antihistaminic SU-4173 (8-methyl-10 [2-dimethylaminopropy] thianaphthindole tartrate); gamma globulin; and Anergex ("special extract of *Toxicodendrum quercefolia*"). Other activities have consisted of advisory services to the Food and Drug Administration, other groups of medical specialists, physicians, and the public, regarding various problems connected with drug therapy or reactions to drugs.

Respectfully submitted,
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Detroit 21, Michigan
Secretary, Committee on Drugs of the Research Council, American Academy of Allergy

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REPORT OF THE RESEARCH COUNCIL OF THE AMERICAN ACADEMY OF ALLERGY FOR 1957-1958

THIS report is divided into two sections: (1) Reports of the individual committees and subcommittees and (2) a report on administrative matters pertaining to the Research Council as a whole.

COMMITTEE REPORTS

A. *Committee on Aeroallergens.*—

1. *Subcommittee on Pollen and Molds:* This group again has conducted its annual extensive survey of pollen and mold counts in many areas of the United States, Canada, and foreign countries. The Committee also answers a large number of inquiries about pollen counts, pollen survey methods, botanical questions pertaining to allergy, and identification of plant and pollen specimens. A number of plant and pollen specimens have been added to the Committee's herbarium. Tinted transparencies of plants important in allergy have been provided at cost to a number of allergists. The Committee presented an exhibit at the annual meeting of the American Academy of Allergy in Philadelphia.

Special projects this year have included sampling in the Lake Tahoe region and work to encourage ragweed control measures in Oregon. Several members of the Committee provided information and stimulation which resulted in a favorable report on ragweed control measures by the City Club of Portland. This appeared to be an important factor in influencing the Oregon state legislative session in 1957 to pass a bill creating an active ragweed control program this past summer. The Committee will write a letter of commendation and encouragement to the appropriate authorities and newspapers in Oregon.

Membership. Oren C. Durham, Secretary for Pollen; Dr. David Merksamer, Secretary for Molds; Drs. Clarence Bernstein, Jose L. Cortes, A. O. Dahl, Bernard Fein, Sidney Friedlaender, Frank Joyce, Eloise Kailin, Dean Moffat, Frank Perlman, Roland Rooks, Bram Rose, Merle Scherr, Bernard B. Siegel, Grace Talbott, Abram Targow, Albert Tocker, Lester Todd, Martyn Vickers, Ralph Voigt, Charles Walton, and Matthew Walzer.

2. *Subcommittee on Dust:* This group has been inactive in the past year. Further progress in individual research is necessary before group projects in this field will be feasible.

Membership. Dr. Francis C. Lowell, Secretary; Drs. Max Samter and Irving W. Schiller.

3. *Subcommittee on Insect Allergens:* The activities of the group and the larger Insect Allergy Study Group have included the following:

(a) Collection of insects for the preparation of allergenic extracts. Evaluation of methods for preparing such extracts.

(b) Collection of information by a questionnaire sent to members of the Committee and study group.

(c) Extracts of eighteen different insects were sent on request to physicians on the condition that clinical reports be returned.

(d) Collection of information in collaboration with entomologists, particularly Dr. Charles Woodworth.

(e) Data obtained by the Committee have been used in part in a paper presented by the Committee's secretary at the 1958 Academy meeting.

Membership. Dr. Frank Perlman, Secretary; Drs. Richard Etter, Alan Feinberg, Frank Rawling, Henry Stevens, Robert Stier, Boen Swinny, and Martyn Vickers.

4. *Subcommittee on Weather:* This group has continued with its major project of attempting to make correlations between clinical data concerning asthmatic patients in widely scattered areas of the country and weather data. Many more active contributors are needed before significant results can be anticipated.

Membership. Dr. Harold S. Tuft, Secretary; Drs. Johnny Blue, M. H. Brodkey, Alan B. Cazort, Armand Cohen, Stanley Cohen, Victor Cohen, Seymour Crepea, Murray Dworetzky, William Eisenstadt, H. M. Farmer, Alan Feinberg, John Fulton, Frank Furstenberg, Morton Hammond, M. Coleman Harris, John Jacobs, Eloise Kailin, Bennett Kraft, Macy Levine, John Mitchell, Dean Moffat, Joseph Noah, Frank Perlman, David Pipes, Edward Seidmon, John Sheldon, W. B. Steen, J. E. Stroh, Joel Teigland, and J. Warrick Thomas.

5. *Subcommittee on Air Processing:* This Committee has answered many inquiries about air filtration devices. It also has obtained a supply of reprints of last year's Research Council report (as it appeared in the JOURNAL OF ALLERGY), as it contains much valuable information about air processing. In cooperation with the Allergy Foundation of America, it is preparing a pamphlet to aid in answering the many inquiries received on this subject. It remains prepared to test air filtering devices, but no requests for testing have been received in the past year.

Membership. Dr. Willard Small, Secretary.

B. *Committee on Contactants.*—This group has functioned in an advisory capacity.

Membership. Dr. Max Grolnick, Secretary; Drs. Rudolf Baer, Merrill Chase, Naomi Kanof, Rudolf Mayer, Samuel Peck, Donald Pillsbury, Adolph Rostenberg, and Marion Sulzberger.

1. *Subcommittee on Testing Procedure:* This group has functioned in an advisory capacity.

Membership. Dr. Rudolf L. Baer, Secretary; Drs. Merrill Chase, Herman Eisen, David Fassett, Rudolf Mayer, Adolph Rostenberg, Max Grolnick, Donald Birmingham, George Curtis, John Fromer, Naomi Kanof, Albert Kleigman, Irvin Kerlan, Morris Leider, Karl Steiner, Raymond Suskind, and Bert Vos.

2. *Subcommittee on Legislation:* This group has functioned in an advisory capacity.

Membership. Dr. Naomi M. Kanof, Secretary; Drs. Samuel Becker, Irvin Kerlan, and Adolph Rostenberg.

3. *Subcommittee on Miscellaneous Excitants:* This group has functioned in an advisory capacity.

Membership. Dr. Morris Leider, Secretary.

C. *Committee on Drugs.*—The report of this Committee was presented on the regular program of the American Academy of Allergy in February, 1958, and is being published in the JOURNAL OF ALLERGY.

Membership.—Dr. Sidney Friedlaender, Secretary; Drs. Murray Albert, Clarence Bernstein, Theodore Bernstein, Joseph Besser, Earl Brown, Seymour Crepea, Alex Friedlaender, Harold Friedman, Frank Joyce, Edmund Keeney, Saul Malkiel, Dean Moffat, Milton Mosko, Samuel Rynes, Irving Schiller, Sheppard Siegal, Bert Schoenkerman, and Walter Winkenwerder.

D. *Committee on Food Allergy.*—This group has been engaged in several activities:

1. Study of the antigenic relationship among members of the fish *(Pisces)* family. Extracts from each of the many subdivisions of the fish family have been prepared. Fish-sensitive sera have been collected, and comparative passive transfer tests have been carried out.

2. Allergy to fresh fruit is being studied by passive transfer tests with selected sera and fruit extracts.

3. In collaboration with the Committee, Dr. Sheldon Cohen has carried out a large number of studies concerning the antigenic relationship of brine shrimp and water fleas (crustaceans) to edible shellfish allergens by agar-diffusion methods with rabbit antisera. By the same method, he also has been conducting studies concerning the presence of antigenic fish proteins in fish liver oils and emanations of cooking fish.

4. An evaluation of food extracts produced by different methods is in progress. Serum has been made available by the Reagin Bank for doing comparative passive transfer tests with the extracts, and medical assistance has been procured to proceed with the testing.

5. The Reagin Bank continues to send and receive sera, and a "Bank statement" has been issued for publication in the Academy's "News and

Notes.'' The bank has collaborated in studies being conducted by this Committee, particularly that mentioned in the preceding paragraph.

Membership. Dr. George I. Blumstein, Secretary; Katherine Bowman, Dr. Sheldon Cohen, Mary Johnson, Dr. Eloise Kailin, Dr. Saul Malkiel, Dr. Bernard Siegel, Mrs. Margaret Strauss-Ballard, Dr. Abram Targow, and Dr. Richard Wiseman.

ADMINISTRATIVE REPORT

The Research Council of the American Academy of Allergy now has been in existence for about a decade. In comparison with the research units of other national clinical societies, the accomplishments of the Council have been substantial. However, there have been criticisms of the relative inactivity of some of the committees and subcommittees of the Council, and at times the rather elaborate administrative structure of the Research Council has resulted in an inordinate amount of administrative work for the results which have been forthcoming. For these and other reasons, an analysis of the Council's activities, objectives, organization, and membership was prepared at the request of the Executive Committee of the Academy and considered at length by them at their interim meeting in October, 1957. It appears that the fundamental problems of allergy will not be solved by committees but by painstaking individual research over a prolonged period of time. The Academy does not have the funds to finance individual research at present, and since the establishment of the Research Council, alternative sources of funds for individual research in allergy have come into being. Working within these limitations, it should not be expected that the major and basic problems in allergy will be solved by the Research Council. At a more superficial level, however, the Council can give valuable service in the areas of group research, exchange of ideas and information, and perhaps in stimulating research. It seems appropriate that the organization and membership of the Council should be patterned to meet these realistic objectives.

The Executive Committee has requested the secretary of the Research Council to prepare some alternative proposals regarding the future of the Research Council. These have been submitted to the *ad hoc* Academy Committee on the Resarch Council for study and a subsequent report to the Executive Committee. The latter has expressed a desire to create closer ties between the Council and the Academy.

Meanwhile, a number of changes in the internal organization and membership of the Research Council were made at the meeting of the Governing Board of the Research Council on Jan. 31, 1958. Seven amendments to the Council's Rules and Regulations were passed. Two of these created a procedure for dropping inactive committees and subcommittees. Other amendments established a three-year limitation on the tenure of committee and subcommittee secretaries. An Advisory Board to the Research Council was created. Another amendment which is of direct interest to all members of the Academy is as follows: ''Members are to be selected from among those persons applying in

writing for membership on the various committees in response to an invitation for such applications to be issued to the Fellows and Members of the Academy by the Secretary of the Research Council no less frequently than once a year. Applications for Research Council membership must be received within two months after the conclusion of the annual meeting of the Academy and must indicate that the applicant is willing to participate in group research projects sponsored by the committee. Committee members will be selected from the applicants on the basis of their apparent potentialities for contributing to the work of the group. Membership on the various committees of the Research Council shall be for a period of one year, but members may *reapply annually* in the manner prescribed above.''

Representatives of the Allergy Foundation of America were invited to the meeting of the Research Council Governing Board and mutual problems were discussed. Apparently the Council can assist the Foundation most in offering materials and information concerning allergy. Communications also were established with the National Institute of Allergy and Infectious Diseases and with the Allergy Committee of the Research Council of the American College of Chest Physicians with the objectives of both offering service and avoiding duplication of effort.

The Governing Board of the Research Council adopted a general policy of opening the committee and subcommittee meetings to all interested members of the Academy. The Reaginic Serum Bank was removed from the Committee on Food Allergy and established as a separate committee of the Research Council. The Subcommittee on Dust was disbanded. The suggestion was forwarded to the Executive Committee that it might be worth exploring the possibility of presenting a prize to the Allergy Foundation of America's undergraduate medical scholar who does the best piece of work each year.

Respectfully submitted,

Kenneth P. Mathews, M.D.

Secretary

Correspondence

March 1, 1955*

TO THE EDITOR:

The research reported by Smith and Rooks in the JOURNAL OF ALLERGY (**25**: 36-45, 1954) represents a beginning of a kind of inquiry which has been needed for a long time. It is unfortunate, however, that they show only the average hourly pollen concentrations for the first and second halves and for all of the ragweed pollen season. Meteorologically, it is quite doubtful whether the days during their period of record could be sufficiently uniform to justify such an averaging process or to assure the validity of the averages obtained.

On the other hand, aside from these considerations, there is the possibility that the important relationships, which one should like to learn about, between the pollen count extremes and the weather are totally obscured by the averaging process. For example, it would seem appropriate to ask what caused the rise of the counts around 12 midnight to 1 A.M. that appears in Fig. 3 of the paper by Smith and Rooks. It is pertinent to observe that hay fever patients probably respond to specific occurrences. When weather conditions are right to produce sufficiently high pollen concentrations at the breathing level of the patient, such an occurrence takes place. If, therefore, one observes *occasions* rather than *averages*, he is probably better able to relate "cause and effect" at least crudely, since weather may properly be regarded as a continuing series of events suitable for correlation with the individual occasions of high pollen concentration.

In addition to these philosophical considerations, which apply quite broadly to the literature on aeroallergens, it is important to point out and correct Smith and Rooks' misinterpretation of Crawford's work (Pub. Health Rep. **64**: 1195, 1949). This is doubly important because the abstract of Crawford's paper (Allergy Abstracts **15**: 66, 1950) also gives an erroneous impression of the results. Crawford observed that a *vertical* current of 1.58 cm. per second was sufficient to lift individual pollen grains of *Ambrosia elatior* from rest. Smith and Rooks have mistakenly reported that ". . . a horizontal air flow of 1.58 cm. per second would be sufficient to overcome the force of gravity and produce airborne pollen . . ." and have given Crawford as reference. Obviously, their subsequent discussion of wind effects is invalidated by this error.

**Editor's Note*: The delay in publication is acknowledged to be in part the consequence of a regrettable, but nevertheless to some extent unavoidable, discontinuity attendant upon a change in editors and to the attempt to learn whether any comment on Dr. Dingle's letter was to appear with it.

In the abstract (loc. cit.) it is reported that Crawford found the specific gravity of ragweed pollen to be 11.3 and that he *computed* the rate of fall of 0.525 feet per second for ragweed pollen. For the record, Crawford *measured* the vertical air current necessary to refloat ragweed pollen grains and called this the fall rate in still air, that is, 1.58 cm. per second = 0.0525 feet per second (not 0.525), and from this, by means of Stokes' law, he *computed* the specific gravity of 1.3 (not 11.3).

To return to the paper by Smith and Rooks, I should like to add a note concerning the magnitude of the effects of humidity upon pollen fall rates. Since it is possible by means of a few simple calculations to place this discussion on a quantitative basis, this appears to be worth while. Starting with the following assumptions, the basis of which is to be found in the literature cited in each case, the analysis follows directly:

(1) At 100 per cent relative humidity the water accumulated by each pollen grain weighs 0.65 as much as the dried pollen grain. (Durham; J. ALLERGY **14**: 455-61, 1943.)

(2) The dried pollen grain is not appreciably spongelike in structure. (Crawford, loc. cit.)

  (a) This is further supported by the often-reported observation that pollen grains shrink upon drying and expand again to near-spheres upon moistening. Such shrinkage and expansion do not characterize a sponge.

  (b) A corollary is that the volume of the moist pollen grain is equal to the sum of that of the dried pollen and that of the water added.

Therefore, a sample of pollen which, when dry, would weigh 1.3 gram and would occupy 1 cm.^{3} (Crawford) will at 100 per cent relative humidity have added 0.845 gram of water occupying 0.845 cm.^{3} additional space. The average density of the moist pollen must then be 1.163 gram per cm.,^{3} and the volume of each grain must be increased by a factor of 1.845.

It follows that the diameter of each pollen grain must be increased by a factor of $\sqrt[3]{1.845} = 1.23$ upon moistening. Using these figures to arrive at a comparison between the Stokes' law fall speed of the moist pollen grains and that of the dry pollen grains, one finds that the ratio is 1.35 to one.

Thus, the increase of fall speed due to moistening is about one-third. If we take Crawford's value for the fall speed of dry pollen as 3.15 feet per minute, this gives a fall speed for moist pollen of 4.25 feet per minute. While this represents an appreciable increase, it still does not amount to a rapid fall rate.

Undoubtedly, the most important factor contributing to cleaning of the air at night is the suppression of atmospheric turbulence. This both cuts off the supply of refloated pollen and dust from the ground surface and allows gravitational settling to operate undisturbed upon the air-borne pollen present. This also, incidentally, must contribute substantially to the repollution of

the air in the morning as low level turbulence again becomes active and refloats large numbers of pollen grains resting loosely upon surfaces of all kinds. Smith and Rooks have, for the first time to my knowledge, pointed up the significance of this refloatation process as the means of extending the hay fever season beyond the season of active pollen production. An important process operating to curtail this extension is the natural deterioration with age and exposure of the pollen contents in terms of antigenicity.

A. Nelson Dingle, Sc.D.
Meteorological Laboratory
University of Michigan

Announcements

Committee on Food Allergy

The Committee on Food Allergy of the Research Council of the American Academy of Allergy is in need of additional sera from persons who are allergic to fish, for continuation of experiments. It would be much appreciated if such sera were sent to the Reaginic Bank at the Jewish Hospital of Brooklyn, 555 Prospect Pl., Brooklyn 16, New York.

Third International Congress of Allergology

The Third International Congress of Allergology will be held in Paris, France, Oct. 19 to 26, 1958. The Congress is sponsored by the International Association of Allergology, under the Presidency of Dr. Samuel M. Feinberg, Chicago, by invitation from the French Allergy Association. The program will include symposia on asthma and emphysema, antibodies, recent clinical advances, biochemical aspects, autoimmune reactions, atopic dermatitis, and socioeconomic aspects. There will be many participants of international renown, including such authorities as Sir Henry Dale, Dausset, Harrington, Forsham, Schild, Sulzberger, Löffler, Kabat, Good, Dixon, Chase, Jimenez-Diaz, and Pasteur Vallery-Radot. Short sectional papers will also be presented, and small round-table luncheon conferences will be led by authorities in several fields.

For travel plans, write to Thos. Cook and Son, Inc., 166 N. Michigan Ave., Chicago, Illinois. For registration and Congress information, write to Dr. B. N. Halpern, 197 Boulevard St. Germain, Paris VII, France.

As of June 1, 1958, 675 doctors have registered.

The Bio-Sciences Information Exchange

The Bio-Sciences Information Exchange is a clearinghouse for current research in the biologic, medical, and psychological sciences. It serves both government and nongovernment granting agencies and investigators associated with recognized research institutions and is supported by the following: Atomic Energy Commission, Department of the Air Force, Department of the Army, Department of the Navy, Public Health Service, Veterans Administration, and National Science Foundation. The Exchange cooperates with the major fund-raising agencies and the private foundations of national scope, and its policies derive from a Governing Board composed of members of the supporting agencies and of the Smithsonian Institution..

You may register your research with the Exchange by writing a summary, of 300 words or less, of your current research problem on a "Notice of Research Project" form and send it to the Exchange. Forms are supplied free of charge upon request. The summary is used as a means of communication. It provides information on work or phases of work which may not be published. The summary should describe the problem clearly, show any direct relationships to other aspects or to broader areas of research, identify

techniques or organisms used, etc. A good summary may be completely and concisely indexed and therefore enjoys the most effective distribution. Reports of progress or results should not be included.

Investigators who wish information concerning specific research problems or a list of research projects being conducted in a given area of investigation may solicit such information by writing or telephoning the Exchange. The Exchange will respond by forwarding pertinent "Notices of Research Projects." The information is available *only* to recognized granting agencies, research institutions, and investigators associated with recognized research institutions. It is not available for publication or publication reference.

The services of the Exchange are provided free of charge.

Obituaries

ISIDORE STANLEY KAHN

ISIDORE STANLEY KAHN of San Antonio, Texas, died on Jan. 26, 1958, of complications of diverticulitis.

He was born in Dallas, Texas, on Aug. 18, 1879. He received his preliminary education at Sach's Collegiate Institute, New York City, and was graduated in 1900 from Harvard University in Cambridge, Massachusetts. In 1904, he received his M.D. degree from Harvard Medical School in Boston. Following an internship at Boston City Hospital, he began practice in Dallas, Texas. Later he practiced in Mexico, before locating in San Antonio in 1912. Dr. Kahn served as a major in the tuberculosis section of the Army Medical Corps during World War I. He retired from active practice in 1953.

Dr. Kahn was a member of the Southern Medical Association, the National Tuberculosis Association, and the Southwest Allergy Forum; he was a Fellow of the American Academy of Allergy, the American College of Allergists, and the American College of Physicians. He was an honorary member of the National Society of Allergy of Spain.

Dr. Kahn published many papers on allergy and is well known not only for his publications but also for his appearances before many of the national and international associations dealing in allergy.

Dr. Kahn and Miss Emma M. Grothaus were married in 1937. She survives, as do two grandsons and three sisters.

His loss will be felt by his numerous friends in the medical profession and especially by those in allergy.

B. S.

HAL MC CLUNEY DAVISON

HAL MC CLUNEY DAVISON died in Atlanta, Georgia, on April 26, 1958, of Hodgkin's disease and complications. He was 67 years of age.

Dr. Davison was born in Woodville, Georgia. He was graduated from Mercer University with two degrees—Bachelor of Arts and Bachelor of Pharmacy—and also won his "M" in football. He received his medical education at Atlanta Medical College (now Emory University) and had further training at New York Post Graduate Hospital and New York Hospital, following which he entered the United States Army Medical Corps and was stationed in Siberia until the end of World War I. He continued there with the American Red

Cross and married Natalia Beklemisheva, who survives him. He is also survived by two sons, Captain Peter H. Davison, now stationed in Germany with the Air Force, and Dr. Alexis H. Davison, now serving his first year's residency at the University Hospital in Charlottesville, Virginia.

Dr. Davison was a pioneer in the study of cerebral allergy, including epilepsy, and his original papers are now source material for students in this field. He was also very much interested in the psychosomatic problems of medicine. A unique aspect of his private practice was lectures to his patients twice a week at his office on this subject, with a question-and-answer period following. He was Chief of Medicine of the Baptist Hospital of Georgia.

Dr. Davison had served as president of his county medical society and in 1957 was awarded an honorary membership in the Southern Medical Association. The same year he was presented with a special resolution from the Fulton County Medical Society expressing profound thanks and appreciation for his dedicated services to medicine. Dr. Davison was also a member of a great many social and business organizations and was active in the alumni affairs of Mercer University, of which he was a trustee.

J. G.